“HISTOMORPHOLOGICAL PROFILE OF GASTRIC ANTRAL MUCOSA IN HELICOBACTER ASSOCIATED GASTRITIS”

DISSERTATION SUBMITTED FOR

M.D. DEGREE EXAMINATION

BRANCH III PATHOLOGY

OF

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

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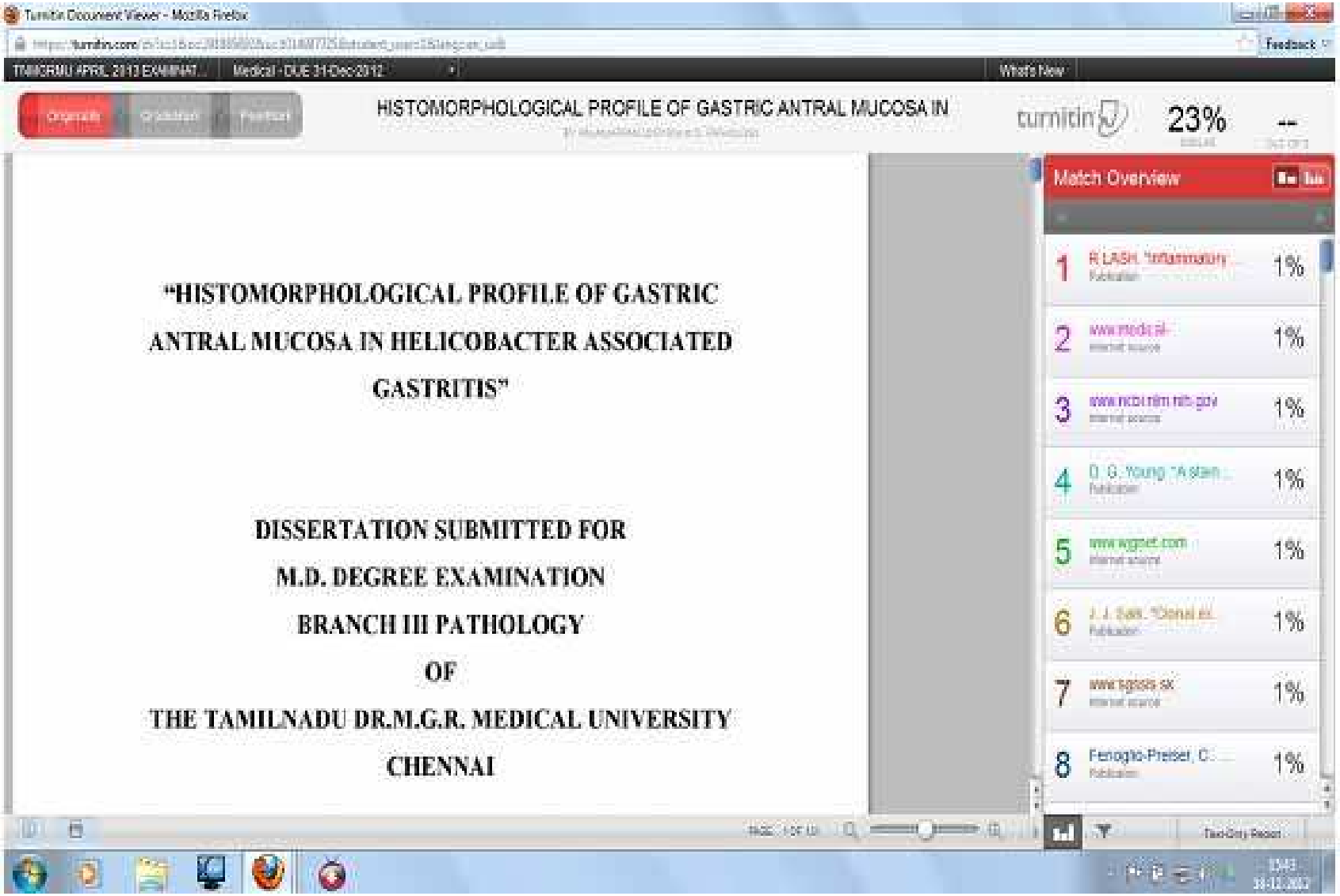
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This is to certify that the Dissertation **"HISTOMORPHOLOGICAL PROFILE OF GASTRIC ANTRAL MUCOSA IN HELICOBACTER ASSOCIATED GASTRITIS"** presented herein by **Dr. V.PALANIAPPAN** is an original work done in the Department of Pathology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch III) Pathology under my guidance and supervision during the academic period of 2010 - 2013.

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I hereby certify that this work embodied in the dissertation entitled "**HISTOMORPHOLOGICAL PROFILE OF GASTRIC ANTRAL MUCOSA IN HELICOBACTER ASSOCIATED GASTRITIS**" is a record of work done by **Dr. V. PALANIAPPAN,** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course in the period 2010-2013. This work has not formed the basis for any previous award of any degree.

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This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr. **V.PALANIAPPAN,** *a* **MD POSTGRADUATE IN PATHOLOGY** *in the Department of* **PATHOLOGY,** *of Tirunelveli Medical College Hospital, Tirunelveli titled* **"HISTOMORPHOLOGICAL PROFILE OF GASTRIC ANTRAL MUCOSA IN HELICOBACTER ASSOCIATED GASTRITIS."** *registered by the* IEC *as* **066/PATHIEC/2011** *dated* **25.02.2011.** *The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.*

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DECLARATION

I solemnly declare that the dissertation titled "**Histomorphological profile of gastric antral mucosa in helicobacter associated gastritis**" is done by me at Tirunelveli Medical College hospital, Tirunelveli.

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LIST OF ABBREVIATIONS

CLO	↱	Campylobacter - like organism
Cag A	↱	Cytotoxin associated gene A
C.jejuni	↱	Campylobacter jejuni
CO_2	↱	Carbon dioxide
C.pylori	↱	Campylobacter pylori
DNA	↱	Deoxyribonucleic acid
GED	↱	Gastric epithelial dysplasia
GI endoscopy	↱	Gastrointestinal endoscopy
HPF	↱	High power field
H&E	↱	Haematoxylin and Eosin
H.pylori	↱	Helicobacter pylori
IARC	↱	International agency for research on Cancer
IL	↱	Interleukin
Ig	↱	Immunoglobin
kDA	↱	Kilo Dalton
MALT	↱	Mucosa associated lymphoid tissue
NUD	↱	Nonulcer dyspepsia
IM	↱	Intestinal Metaplasia
PAS	↱	Periodic acid Schiff
PMN	↱	Polymorphonuclear neutrophil
RT-PCR	↱	Reverse transcriptase- Polymerase chain reaction
TNF	↱	Tumor necrosis factor
Vac A	↱	Vacuolatingcytotoxin A
WHO	↱	World Health Organization

CONTENTS

INTRODUCTION

Since 20th century, there were many reports of spiral organisms being isolated from the stomach of human beings and the possible role of these organisms in any human gastric disease. It was culmination of many years of effort by J. Robin Warren and Barry J. Marshall in Perth, Australia. With the isolation of H.pylori, the flood gates opened to a new understanding of gastro duodenal pathology and its implications in the management of peptic ulcer disease.[1]

It is a bacterial pathogen infecting the gastric antrum of half the population worldwide. Helicobacter pylori(H.pylori) infection has found to be associated with acute and chronic gastritis,non-ulcer dyspepsia, peptic ulcers, gastric adenocarcinoma and gastric MALT associated lymphomas.[2]

Colonization of stomach by H.pylori and chronic active gastritis has a cause and effect relationship. Early detection and eradication of H.pylori infection is very essential because it not only cause healing of gastric inflammatory lesion but also helps in reversal of precursor lesion which results in carcinoma such as gastric atrophy and intestinal metaplasia. [3]

The Sydney system for the classification of gastritis emphasises the importance of topographical, morphological and etiological information. This system was revised at the Houston Gastritis Workshop held in 1994.

The histological severity of H.pylori density, inflammation, activity, atrophy and intestinal metaplasia were graded according to the updated Sydney system.[4]

Endoscopic biopsy allows detection of H.pylori, which determines treatment for peptic ulcer disease.

For most studies histology is the standard for detection, because the objective is determination of whether the organisms are present and whether there is gastritis.

The epithelial changes in H.pylori colonized gastric mucosa can be recongnised with routine H&E sections and it helps as a indicator to notify the presence H.pylori in gastric mucosa .

As effective specific treatment for H.pylori associated gastroduodenal disorders is already in vogue,the identification of the organism has become mandatory in antral biopsies.

The present study was planned to analyse the spectrum of the light microscopic histopathologic changes in gastric mucosa colonized by H.pylori in patients presenting to the outpatient department of medical gastroenterology.

AIMS AND OBJECTIVES

1. To evaluate the spectrum of light microscopic histopathological changes in gastric mucosa in patient with active chronic gastritis.
2. To detect the H.pylori organism using Light green stain and compare the staining qualities with that of Warthin – Starry Silver stain.
3. To correlate the bacterial load with the histomorphological changes.
4. To analyse the histomorphological changes of esophagus in the same cases.

REVIEW OF LITERATURE

EMBRYOLOGY OF STOMACH:

The embryonal stomach is derived from the primitive foregut. At the end of 5th gestational week, the embryonal stomach undergoes a 90 degree rotation around its longitudinal axis, so that left side faces forward and the right side faces toward the back.This determines that the left vagus nerve is located anteriorly in postnatal life whereas the right vagus nerve innervates mostly the posterior aspect of the stomach. At the time of such rotation, the posterior wall grows more rapidly than the anterior, thus giving rise to major and lesser curvatures.[5]

In the early stages, the stomach is situated in the midline. However, later on, the caudal portion is mobilized upward and to the right, constituting the antropyloric portion.The cephalic portion, on the other hand, rotates toward the left and slightly downward, becoming cardia.[5]

ANATOMY AND HISTOLOGY:

Anatomically, stomach is divided into four regions: the cardia, fundus, body(corpus) and pylorus (pyloric antrum). Cardia is a small area of mucus secreting glands surrounding the entrance of the oesophagus. It measures only a few millimetres or may be incomplete or absent altogether.[6]

Mucosa of the fundus and body of the stomach consists of straight tubular glands that extend upto level of muscularis mucosa and gastric

pits that occupy about one quarter thickness of the gastric mucosa and has gastric glands opening into it. The gastric glands contain a mixed population of cells, surface mucus cells, neck mucus cells, parietal or oxyntic cells, chief cells (peptic or zymogenic cells), neuroendocrine cells and stem cells.[6]

Gastric luminal surface and part of the gastric pits are covered by surface mucus cells .Neck mucus cells are seen between the parietal cells in the neck and base of the gastric glands. Parietal cells are distributed along the length of the glands and they are identified with following histological features showing large round cells having dense eosinophilic cytoplasm with centrally placed nucleus whereas Peptic cells are recognised by their dense basophilic cytoplasm with condensed basally located nucleus. Neuroendocrine cells are found in the base of the gastric glands. Stem cells are found in the neck of gastric glands whereas Lamina propria is loose vascular and scanty.[6]

The submucosa is also loose like that of lamina propria but it is distensible and contains large blood vessels. Muscularis propria composed of two layers inner circular and outer longitudinal layers. The serosal layer, which covers the peritoneal surface, is thin.[6]

Pyloric glands are branched and coiled and gastric pits occupy about half the thickness of the pyloric mucosa. The glands are lined almost exclusively by mucus secreting cells, a small number of parietal

cells and neuroendocrine cells that secrete gastrin and thus are called G cells.[6]

HELICOBACTER PYLORI –HISTORICAL PERSPECTIVE :

Marshall BJ et al (1984) imprinted in his study that, for the past 45 years spiral bacteria in stomach have been repeatedly observed, reported and forgotted. In 1890s, Bizzozero described the spiral bacteria in dogs.[7]

Dooley CP mentioned in his study that early review shows the presence of spiral shape organism in the stomachs of dogs in 1893. It was also reported in rats and cats in 1896. Autopsy study by Doenges in 1938 found the prevalence rate of 43% for spiral organisms in the human stomach, but unfortunately he did not detect a relation between the presence of the organism with its relation to various gastric diseases.[8]

1906 Cheng ML et al in their study mentioned that in gastric spiral organisms was identified in vomitus from a patient with gastric carcinoma.[9]

Marshall BJ et al in 1940,mention that Freedburg and Baron stated that “spirochetes” could be found in up to 37% of gastrectomy specimens, but examination of gastric suction biopsy material failed to confirm this finding.[10]

Buckley MJM et al in their study mentioned that in 1950, Fitzgerald and Murphy Studied about urease in the resected stomachs of

patients with gastric ulceration. In 1968, the bacterial source of gastric urease was confirmed.[11]

Marshall BJ et al mentioned in his study in 1975 that spiral bacteria have strong association with gastritis about 80% and he studied this in gastric resected specimens from patients with gastric ulceration.This spiral bacilli were said to be Pseudomonas at first, possibly a contaminant and the bacteria were forgotten again.[10]

Buckely MJM et al in their study mentioned that in 1979, spiral bacteria were observed in the luminal surface of epithelial cells of gastric ulcer patients.

Hellstrom PM et al in their study mentioned that in 1982, Australian doctors Barry J. Marshall and J. Robin Warren discovered the bacteria and found that the appearance of this bacteria was similar to that of Campylobacter genus and primarily named the bacteria as Campylobacter pyloridis.[11]

BACTERIOLOGY:

The organism shares the features of Campylobacter organism such as micro-aerobic, curved, gram-negative ,so they were named as Campylobacter pyloridis initially. Later they found that it is a motile bacillus with multiple polar flagella and they distinguish it from other campylobacter species by its surface proteins, the unusual fatty acids that compose its cell wall and its deoxyribonucleic acid (DNA) composition.[12]

Goodwin CS et al (1993) and Windsor HM et al (2000) in their study mention that the new name Helicobacter was published in October 1989 (Helico = curved, bacter =staff). According to Kusters JG et al, in 1989, a new genus name was suggested 'Helicobacter' reflects the two morphological appearances of the organism, helical in vivo but often rod like in vitro and that this organism is distinct from members of the genus Campylobacter.[13]

H.pylori organism have following characteristic features for their identification they are tiny spiral form organism measuring 1 to 3 microns in length and 0.3 to 0.6 microns in width with unipolar, multiflagellate. Under unsuitable environment the spiral shape H.pylori organism will undergo transformation into coccoid forms and this Coccoid forms measures range from 0.4 to 1.2 microns in diameter and ultrastructurally appear as U shaped bacilli with ends joined by a membranous structure. H.pylori usually present in surface of the gastric mucosal lining or sometimes within the epithelium or within the lumen of the gland. In haematoxylin and eosin (H&E) stained section it appears as faintly eosinophilic organisms. Sometimes it is difficult to identify the H.pylori organism because it is easily masked by abaundant mucus, cellular membranes of gastric epithelial cells and contaminating oropharyngeal mucosa or other foreign material introduced by the endoscope. H. pylori

are microaerophilic, growing best in an atmosphere of 5% oxygen with 5-10% CO on the blood containing media.[14]

Its helicoidal shape and high motility due to action of multiple flagella allow it to cross the thick layer of mucus and stay beneath it, thereby making the successful colonization of the human stomach possible, where gastric acidity and peristalsis normally inhibit bacterial coloniszation.[15]

RESERVOIR OF H.PYLORI INFECTION:

Human stomach is the natural reservoir of the H.pylori infection. But it may also detected in the laboratory animals such as monkeys and cats when they are infected with H.pylori organism.The DNA of H.pylori were found to be present in 60% milk samples and 3% sheep tissue samples. [16]

Intestinal tract of house flies may harbour H.pylori infection but there is no evidence of transmission to human from them were detected till date.[17]

SURVIVAL OF H.PYLORI IN THE STOMACH:

Eaton and Krakowa mention that urease is essential for the survival of H.pylori in the acidic environment .Thus for initiation of infection urease must be neeed.H.pylori make fit to reside in the acidic medium by means of splitting urea by secreating large amount of urease into the

microenvironment .Compare to other bacteria ,H.pylori has urease in their intact cell membrane but other bacteria will have it in the cytoplasm.[18]

Normally H.pylori has its urease in the cytoplasm but they may be actively adsorbed into the cell membrane . The presence of supramolecular structure in Urease enzyme make the H.pylori to survive in acidic environment. H.pylori urease has both alpha and beta subunit along with internal cavity which is filled by tissue fluid but it will be filled by urease during the time of requirement.[19]

INCIDENCE AND TRANSMISSION:

H.pylori infections occur worldwide, although there are marked geographic variations in their incidence. The prevalence of H.pylori infections in adults ranges from <15% in some populations to virtually 100% in less well-developed areas. There are also substantial variations among different ethnic groups in the same geographic locale. Because of the low socioeconomic condition H.pylori infection affects the people earlier in developing countries than the developed countries of the world, so thereby prevalence of H.pylori infections correlates well with lower socioeconomic status.[20] The human stomach is the primary reservoir for the organism and it is transmitted via an oral route and possibly via a gastric oral and faeco oral route .[21] The infection is easily passed from one family member to another, particularly in areas of dense housing. Children under the age of 5 are most susceptible to H.pylori infections.

H.pylori infections are present in gastric biopsies of 16.8% to 55% of children with abdominal pain, upper gastrointestinal symptoms and histologic evidence of acute and chronic gastritis.[20]The infection prevalence in developing countries can be as high as 75% by age 25.

There has been a recent decline in the incidence of H.pylori infections in the developed world, largely due to improved living conditions and a decrease in living density and family size. Even though the prevalence of H.pylori infections is currently decreasing, at least 50% of the world population is infected with the organism.[22]

Recurrent infections are usually a persistent infection rather than the acquisition of new infections. Infection with more than one H.pylori strain can also occur.

When AIDS patients become infected, the disease may exhibit particularly virulent characteristics and large numbers of organisms may be present.

PATHOGENESIS:

H.pylori is highly adapted to occupy a special ecologic gastric niche with unique features that allow it to enter the mucus of the mucosal barrier, attach to the epithelium, evade immune responses, proliferate and colonize the gastric mucosa. The eventual outcome of H.pylori infections reflects strain-specific, environmental and host-related factors. After they are ingested, organisms must evade the bactericidal activity of the gastric

lumen and enter the mucous layer. Corkscrew like bacterial movement and enzyme production (particularly urease and lipase) are important early in the infection.Bacterial proteases digest gastric mucin facilitating bacterial movement and urease protects the H.pylori from the luminal acid by creating an alkaline microenvironment around the bacterium.[23,24]

H.pylori bacteria normally reside in the unstirred layer of gastric mucus. They wind down to the epithelial surface, moving easily through the viscous environment above it, to attach themselves to the apical membranes of foveolar cells . Bacterial adhesins recognize cell surface specific proteins facilitating epithelial colonization. The best characterized adhesin, BabA, is a 78-kD outer bacterial membrane protein that binds to the fucosylated Lewis B blood group antigen.[25] The Lewis blood group terminal carbohydrate structures are present on the ends of MUC1 carbohydrate side chains as well as on secreted mucins. MUC1 is highly polymorphic and evidence suggests that functional allelic differences affect infection susceptibility.[26]H.pylori bacteria also bind to MUC5AC, a major mucin produced by foveolar cells. Bacteria unable to adhere to the epithelium are rapidly cleared from the mucosa. H.pylori bacteria preferentially attach at or near intercellular junctions, penetrating the junctional complexes moving down along the lateral cell membranes. This disrupts intercellular tight junctions between viable cells, allowing luminal contents, including acid, to flow between the cells.

Most H.pylori strains secrete the vacuolating cytotoxin (VacA). This toxin inserts itself into the epithelial cell membrane and forms a hexameric anion-selective, voltage-dependent channel through which bicarbonate and organic anions can be released, possibly providing H.pylori bacteria with their nutrients. [27]VacA also inhibits T-lymphocyte activation. Most H.pylori bacteria possess the cag pathogenicity island (cag PAI) that contains 29 distinct genes. Some of these genes facilitate translocation of the CagA protein into foveolar cells.[28] Once in the cells, CagA is phosphorylated and binds to the SHP-2 tyrosine phosphatase,leading to host growth factor like cellular responses, cytokine production and cell proliferation.[29] These cytokines mobilize leukocytes to areas of immune challenge . CagA also plays a major role in disruption of the apical junctional complexes. CagA-positive strains associate with increased epithelial cell apoptosis.[30]

The oipA (outer inflammatory protein) gene encodes one of the outer membrane proteins and is an inflammation-related gene near, but not in, the CagA PAI. oipA functional status correlates with clinical presentation, H.pylori density and gastric inflammation. OipA and the cag PAI are both necessary for full activation of the IL-8 promoter.Nitric oxidase synthase and cyclooxygenase-2 are induced by H.pylori infections; these enzymes modulate the inflammatory responses.[31]

HOST RESPONSES TO HELICOBACTER PYLORI :

H.pylori infections cause gastric inflammation (gastritis) in almost all infected persons, although the severity of the changes varies among individuals. The injury results from both the infection and its associated inflammation. Bile reflux and dietary irritants may further enhance the deleterious bacterial effects. Additionally, anti-H.pylori antibodies that cross-react with the gastric mucosa induce further damage. Some H.pylori infected patients develop an autoantibody response directed at the H^+-K^+-ATPase pump in parietal cells leading to atrophy of the corpus.[32]

Initially neutrophils are recruited to the infected site, followed by the recruitment of T and B lymphocytes, plasma cells and macrophages. The neutrophilic infiltrate and mononuclear phagocytic activation may be facilitated by bacterial urease production and induction of nitric oxide synthase and cyclooxygenase.[33] H pylori infections generate significant cellular and humoral responses via antigenic stimulation of mucosal monocytes and T cells. The inflammatory cells produce numerous cytokines (TNF, interferon and interleukins 1, 6 and 8), prostaglandins, proteases and reactive oxygen metabolites that cause epithelial necrosis and mucosal injury. IL-8, a potent neutrophil-activating chemokine expressed by gastric epithelium, plays a central role in the inflammatory response.[34] H pylori bacteria containing the cag PAI induce a stronger IL-

8 response than cag-negative strains. Some cytokines promote leukocyte adhesion to endothelial cells and others recruit additional leukocytes to the infected site. Mediators of local humoral responses, such as mucosal IgA, attract eosinophils, which then degranulate. Stimulated B cells differentiate into IgM, IgA and IgG antibody producing cells.[35] IgA promotes complement-dependent phagocytosis and H.pylori killing by polymorphonuclear neutrophils (PMNs). Secretory IgA synergizes with IgG to promote antibody-dependent cell-mediated cytotoxicity induced by PMNs, monocytes and lymphocytes. High anti- H.pylori IgG antibody levels correlate with severe antral gastritis and dense H.pylori antral colonization.[36]

H.pylori infections result in hyposecretion, hypersecretion or normal acid secretion, depending on disease stage. Hypochlorhydria develops when the gastritis extends proximally to involve and destroy the oxyntic mucosa. Acid secretion increases via several mechanisms.[37] Patients with an increased parietal cell mass and hyperchlorhydria exhibit antral restriction of the gastritis because the high acid levels protect the corpus from bacterial adhesion and inflammation.

SIGNS AND SYMPTOMS:

H.pylori infected person at early period doesn't show any symptoms .During acute infection patient develops abdominal pain and nausea. Later during chroninc gastritis patient develops abdominal pain, nausea, bloating, blenching and vomiting. H.pylori related inflammation in gastric antral mucosa results in duodenal ulcers whereas inflammation in corpus results in gastric ulcer and gastric carcinoma.[38]

HISTOMORPHOLOGICAL CHANGES IN H PYLORI INFECTED GASTRIC MUCOSA :

1.NEUTROPHIL INFILTRATION

Infiltration of neutrophils in lamina propria of the stomach indicates the active component of gastritis which indicates the release of inflammatory mediators in sustained manner. Detection of neutrophil infiltration helps in differentiation of various types of gastritis. Abundant infiltration of neutrophils in lamina propria were seen in acute phase of infections e.g., phlegmonous gastritis. Moderate to severe infiltration of neutrophils were seen in active phase of Helicobacter-induced gastritis, whereas minimal infiltration of neutrophils in lamina propria is seen in acute hemorrhagic gastritis secondary to chronic alcoholism and use of nonsteroidal antiinflammatory drug.[39]

2.MONONUCLEAR INFILTRATION

Mononuclear infiltrates such as lymphocytes and plasma cells were normally seen in antral mucosa as few numbers but it is completely absent in corpus of the stomach. It is advised to diagnose gastritis in the presence of five or more clusters of mononuclear cells in the lamina propria or else as a diffuse infiltrate.

Infiltration of lymphocytes , plasma cells and mast cells will not be seen normally in gastric mucosa but their presence under abnormal condition strongly suggest that chronic H.pylori gastritis but in autoimmune gastritis, the infiltrate is diffuse, predominantly plasma cells and lymphocytes were seen in mucosa and submucosal layers.[39]

3. LYMPHOID AGGREGATES AND FOLLICLES

In stomach small lymphoid aggregates were seen in corpus near muscularis propria or else at the base of the lamina propria. But in pathological condition such as H.pylori gastritis, large lymphoid aggregates with germinal centers were observed. It indicates the presence of H.pylori in gastric mucosa. In endoscopy presence of multiple nodularity indicates presence of lymphoid follicles which indicates H.pylori induced follicular gastritis.[40]

4. EOSINOPHIL INFILTRATION

In normal person only scattered eosinophils were be identified in lamina propria of gastric mucosa. But in case of H.pylori gastritis few eosnophilic infiltrates will be present along with plasma cells and lymphocytes .Compare to adults, childrens have modereate to severe eosinophilic infiltrtate in H.pylori gastritis.[41]

5. MUCOSAL HYPEREMIA AND EDEMA

Endoscopically mucosal hyperemia and edema indicates the presence of H.pylori gastritis which is due presence mast cells abaundantly in gastric mucosa but it may also be seen in chemical induced gastritis .[42]

6. SURFACE EPITHELIUM DEGENERATION

Any injury to gastric mucosa will results in surface epithelium degeneration which may results in gastritis. Surface epithelial injury is mainly due to chemical injury caused due to bile reflux, ethanol etc. and also due to H.pylori. This surface epithelial Degeneration may leads to change in columnar epithelium into cuboidal epithelium along with depletion of mucin. This findings were seen even when the H.pylori in few numbers in gastric mucosa. In epithelial regeneration the epithelial cells arranged in one over the other in piling up fashion at the surface of the gastric mucosa which is a well known feature of H.pylori associated gastritis.[43]

7. SURFACE EROSION

Severe epithelial injury and necrosis of gastric mucosa leads to Surface erosions. Surface erosion usually confined to muscularis mucosae. Endoscopically H.pylori associated surface erosion will be elevated from the surface of the mucosa. The erosion usually filled with fibrinoid necrosis containing neutrophils and cellular debris and surrounding margin show hyperplastic and regenerative changes of the epithelium.[44]

8. FOVEOLAR ("PIT") HYPERPLASIA

Increase exfoliation of surface epithelium of gastric mucosa will occur in H.pylori associated gastritis .In order to compensate the loss , gasrtric pit epithelial cell get proliferated which leads to elongation and tortuosity of the gastric pit which atlast result in Foveolar hyperplasia. Other important findings of foveolar hyperplasia include the presence cuboidal epithelial cells having hyperchromatic nuclei with increase nuclear cytoplasmic ratio along with features of upper pit mitoses and mucin depletion.[45]

9. INTESTINAL METAPLASIA

Gastric intestinal metaplasia (IM) is quite common. It is believed to represent a response to chronic injury, often caused by H.pylori infections. Intestinal metaplasia has been implicated in the development of both gastric and esophageal carcinoma. Intestinal metaplasia usually

starts at junction between the antrum and corpus in a patchy and multifocal fashion after that it spread both proximally and distally to involve the entire antrum and fundus of the stomach. The areas of Intestinal metaplasia increase with patient age and often become confluent, replacing large areas of the gastric mucosa. Intestinal metaplasia more frequently coexists with gastric cancer than with gastric ulcer, but it shows the same distribution when associated with either condition.

In Intestinal metaplasia the cells that normally line the gastric mucosa (surface epithelium, foveolar epithelium and glands) are replaced by an epithelium resembling that of the small or large intestine. The earliest metaplastic changes consist of the appearance of mucin-negative absorptive enterocytes with a brush border alternating with Alcian blue positive goblet cells. In young individuals with less extensive IM, the metaplastic glands resemble normal small intestinal epithelium. Initially, only the epithelial type changes, but later the mucosal architecture acquires a small intestinal villiform architecture, often containing Paneth cells at the base of the pits. Paneth cells in areas of IM do not have the same uniform distribution seen in the intestine. In some cases, they are limited to the antral corpus border and are lacking in IM in the distal stomach. They may lie in the superficial portions of the metaplastic

gland; ultrastructurally some Paneth cells contain both Paneth cell granules and mucinous vacuoles .

Goblet cells are easily seen on H&E-stained sections. However, an Alcian blue/PAS stain is commonly used to identify the goblet cells since it stains all acidic mucins blue-purple and neutral mucins magenta and is easy to perform and interpret. These cells have a complete switch in their differentiation program from a gastric to an intestinal phenotype and they have been termed small intestinal, complete, or type I Intestinal metaplasia.[46]

10. ATROPHY

Loss of gastric glands leads to atrophy. If there is any injury to gastric mucosa with whatever the etiology may be, it undergo regeneration into normal epithelium or it may leads to change over of native glands into metaplastic glands.

When injured glands fail to undergo regeneration ,the lamia propria will be replased by extracellular matrix and fibroblast which results in loss of function of the gastric epithelium and it is termed as atrophy.The adjacent gastric glands will undergo pyloric metaplasia or intestinal metaplasia. Pyloric metaplasia is named because metaplastic glands resembles to that of normal pyloric glands and intestinal metaplasia is named because the glands have absorptive intestinal epithelial lining with

goblet cells. In autoimmune gastiritis immune mediated destruction occurs in the fundic gland epithelium which result in atrophy.[47]

RELATIONSHIP OF HELICOBACTER PYLORI TO GASTRIC DISEASES:

H.pylori organism plays a significant role in the genesis of several gastric diseases. Patient outcomes reflect differences in host susceptibility, organism virulence or both. The development of gastritis, ulcer and gastric cancer all involve an interplay between environmental, host, genetic and microbial factors.[48]

HELICOBACTER PYLORI RELATED DISEASES

The following are some of the H pylori associated gastric disease entities[48]

- Acute gastritis
- Chronic gastritis
- Chronic active gastritis
- Follicular gastritis
- Atrophic gastritis
- Lymphocytic gastritis
- Granulomatous gastritis
- Gastric duodenal ulcer
- Autoimmune gastritis
- Hyperplastic polyp

- Intestinal metaplasia
- G-cell hyperplasia
- Gastric adenocarcinoma
- Gastric Lymphoma(MALT)
- Menetrier disease

H.PYLORI RELATED GASTRITIS :

H.pylori bacteria preferentially colonize the antrum, but they may infect any part of the stomach where they cause gastritis. When treated, the bacteria migrate from the antrum to the corpus with decreasing activity of the antral gastritis. Infections with vacA-positive H.pylori strains result in acute gastritis with cytoplasmic swelling and vacuolization, other changes were includes micropapillary changes, mucin loss, erosion of the juxtaluminal cytoplasm and desquamation of surface foveolar cells. Regenerating cells form a multicellular layer with indistinct intercellular borders, creating syncytial polypoid excrescences . Marked neutrophilic infiltrates appear in the mucous neck region and lamina propria in early acute gastritis; when severe, they aggregate in the pit lumens to form pit abscesses. The mucosa is elevated or expanded slightly due to the lymphoplasmacytic cell infiltrate in the superficial lamina propria. At this point the lesion can be termed chronic active gastritis or active chronic gastritis. Eosinophils may also be present. The regenerative pit bases are characterized by mucin loss, cytoplasmic

basophilia, increased mitoses and hyperchromatic nuclei that are sometimes severe enough to mimic dysplasia. If the pits and glands appear parallel to one another with intervening lamina propria, in that occasion pathologist should think twice before making a diagnosis of carcinoma, even in the presence of severe glandular or cellular atypia.

Both the neutrophils and the bacteria destroy the epithelium, causing the mucous neck cells to proliferate in an effort to replace the dying cells. Other changes in severe infections include epithelial cell dropout, microerosions, larger erosions and ulcers. Erosions forming in the setting of H.pylori infections typically lack the homogeneous eosinophilic necrosis seen in patients with stress ulcers or aspirin or NSAID related ulcers. The acute foveolitis may associate with an epithelial alteration known as the (clear) cell change. Malgun cells have enlarged euchromatic nuclei, abundant cytoplasm and increased expression of proliferating cell nuclear antigen (PCNA) and cytokeratin 8, indicating that they are mitotically and metabolically active. Malgun cells may be morphologic indicators of genomic damage and repair.[49]

H.pylori eradication causes rapid neutrophil disappearance. Eosinophils disappear more slowly. The surface changes reverse rapidly and the epithelial cells acquire their normal shape and spatial organization within a few days of H.pylori eradication. However, any atrophy that had

developed remains, as do the lymphoid aggregates. These features become a permanent component of the once-infected gastric mucosa.

In quiescent superficial gastritis, the acute inflammation, edema and vascular congestion disappear and the epithelium returns to normal. However, the lamina propria contains increased numbers of mononuclear cells. Chronic superficial gastritis progresses to the next stage, chronic atrophic gastritis, over a period of 15 to 20 years.[50]Since chronic gastritis develops as a patchy process, all stages in the evolution of chronic gastritis often coexist in a single stomach leading to the term multifocal atrophic gastritis.

Lymphoid aggregates appear and sometimes lymphoid follicles develop. These are located deep in the mucosa, near the muscularis mucosae. When lymphoid follicles develop, with or without follicular centers, the lesion is termed follicular gastritis.[51] Antral lymphoid follicles can become quite prominent, sometimes causing mucosal nodularity, especially in children.[52] The lymphoid aggregates represent an immune response to the bacteria. Their presence provides a useful marker for H.pylori infections. Their number may decrease when the H.pylori infections are treated.

Granulomatous gastritis develops in approximately 1% of H.pylori infected patients, usually in patients with small numbers of organisms. The granulomas develop late in the disease, after the host has become

sensitized to the organism. Antibody-coated bacteria ingested by macrophages may stimulate a histiocytic response .[53]

Diffuse antral gastritis (DAG) is often considered to represent part of the peptic ulcer disease spectrum with antral and duodenal ulcers since it associates with increased gastrin, acid and pepsin secretion.[54]The hyperacidity creates a hostile environment for H.pylori bacteria, restricting them to the antrum. The gastritis is characterized by an intense antral mononuclear infiltrate consisting of mature lymphocytes and plasma cells. Follicular gastritis is common. The epithelium may appear mucin depleted and there may be pit elongation.

Occasionally H.pylori infections lead to the development of enlarged gastric folds in the gastric body, creating an endoscopic pattern suggestive of a hypertrophic gastritis/ gastropathy.[55] The H.pylori induced mucosal fold thickening is termed giant fold gastritis. This differs from Menetrier disease in that the mucosa is thinner in giant fold gastritis and there is less foveolar hyperplasia.[56]

SYDNEY SYSTEM:

In view of avoiding the diagnostic confusion in gastritis. In 1990 a workshop was conducted in World Congress of Gastroenterology in Sydney to establish guidelines for a new classification of gastritis based on a grading system called the "Sydney System". In 1994 it was modified at the workshop held at the Houston.

The Sydney System of grading and classification of gastritis is introduced in aim of producing a standardized approach to the histological interpretation of gastric biopsies. Its importance lies to provide a universal language among the pathologist to give diagnosis.

Sydney system has evolved a new classification of gastritis which is simple,comprehensive and easy to apply(Fig.A).[57]

Sydney system consists of two divisions

1. Histological
2. Endoscopic

The backbone of Histological division is topographical distribution of abnormalities of gastric antrum alone, gastric corpus alone, or both pangastritis.

Histological division of Sydney system consists of three parts:

- Aetiological (prefix)
- Topography (Core)
- Morphology (Suffix)

Morphologically, the Sydney system recognizes only three types:

1. Acute gastritis
2. Chronic
3. Special forms

Variables in morphological aspect pertinent to chronic gastritis are as follows:

Normal mucosa:

Contains scattered mononuclear cells, lymphocytes and plasma cells with occasional lymphocyte aggregates in the corpus, no granulocytes.

Acute / Chronic:

Acute gastritis, if neutrophils are dominant. Chronic gastritis if mononuclear inflammatory cells are dominant.

Inflammation:

Refers to presence of inflammatory cells in lamina propria, distribution of which is disregarded in this classification.

Atrophy:

Refers to loss of gastric glands. Old classification as chronic atrophic gastritis is disregarded and inflammation and atrophy are assessed independently.

Activity:

Refers to presence of neutrophilic granulocytes in the lamina propria, in intraepithelial sites or both.

Intestinal metaplasia:

Individual pattern of intestinal metaplasia are classified as type i, ii and iii may be commented on, but are not graded.

Helicobacter pylori:

Presence of Helicobacter pylori is commented upon using a simple stain and not a silver stain.[58]

REVISED SYDNEY CLASSIFICATION [59]

1. Based on site

Antrum, Body& other

2. H pylori:

It is graded as absent when there is no H.pylori were detected in gastric mucosa, in case of mild grade one to three bacteria in pits or on the mucosal surface should be noted , moderate means presence of organism with a layer of bacteria or marked means presence of organism as clumps.

3. Chronic inflammation:

Usually in normal person few number of lymphocytes and histiocytes were present in the lamina propria of gastric mucosa, in case of mild inflammation there should be presence of ten cells more than normal in high power field, in moderate inflammation presence of eleven to twenty cells more than normal and marked inflammation should have twenty cells more than normal.

4. Acute inflammation:

If there is no neutophils in the lamina propria it is termed as normal, presence of 5 neutrophils fall under mild acute inflammation,

presence of 5 to 10 neutrophils comes under moderate and presence of more than 11 neutrophils in the lamina propria along with pit abscess comes under severe acute inflammation.

5. Atrophy:

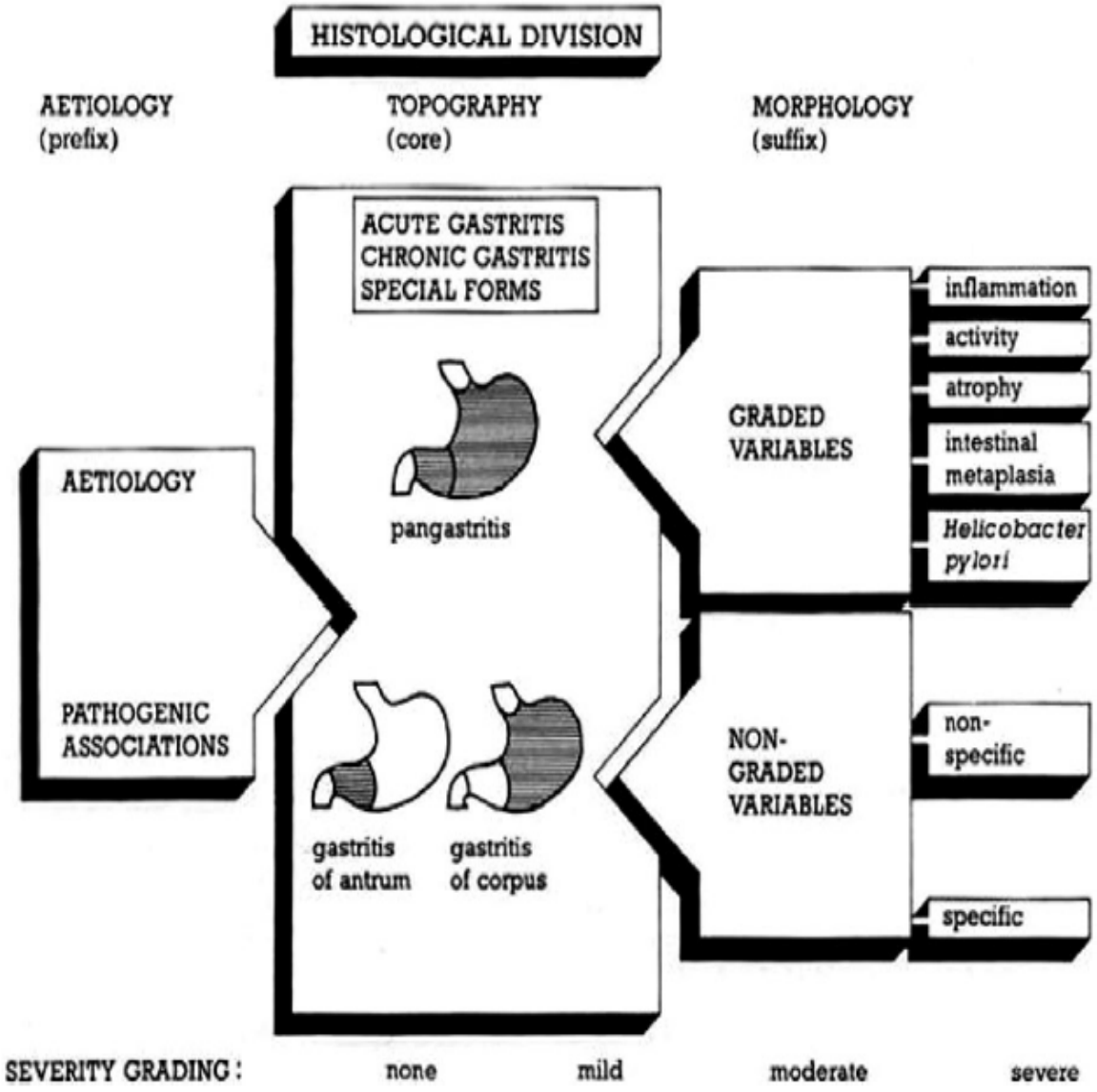
Loss of glands termed as atrophy and it is graded as absent, mild, moderate and marked according to the visualanalog scales.

6. Intestinal metaplasia:

It has two types the first one is incomplete metaplasia in which the gastric glands are replaced into metaplastic glands which shows presence of goblet cells, brush bordercells and Paneth cells and graded as absent, mild, moderate and marked according to the visual –analog scale.

In Incomplete intestinal metaplasia the glands shows the presence of colonic mucosa and goblet mucous cells. Complete intestinal metaplasia resembles small intestine and they shows presence of absorptive brush border cells as well as Paneth and goblet cells. [59]

SYDNEY SYSTEM OF GRADING AND CLASSIFICATION OF GASTRITIS(Fig:A)



NON ULCER DYSPEPSIA:

One of the most common gastrointestinal problem encountered by clinician is Non ulcer dyspepsia (NUD). Non ulcer dyspepsia is defined as complex symptoms in the upper gastrointestinal tract that present for 4 weeks without any structural and biochemical alteration. The Rome consensus committee held in 1991 were establish Diagnostic criteria and later it was updated in 1998 in Vienna. Non ulcer dyspepsia usually present with multifactorial etiology, thereby different mechanisms result in different subtypes of disease, which have been subgrouped on the basis of the predominant symptom type – ulcer like, reflux like and dysmotility like.

Non ulcer dyspepsia were more commonly infected with H.pylori organism compared to the gastritis or peptic ulcer disease patient. Non ulcer dyspepsia patient were more prone for H.pylori infection When compared to asymptomatic person .[56]

According to Rosenstock S et al (1997), studies certain symptoms of non ulcer dyspepsia such as bloating and belching give a clue clinically for the presence of H.pylori in the gastric mucosa.[57]

Tally NJ (1999) mention that 40-90 present of the patient with non ulcer dyspepsia bear H.pylori infection.[58]

Varasa TA et al (2008) observed a that early detection and eradication therapy of H.pylori gives more benefit for those patient with

non ulcer dyspepsia when compared to symptomatic treatment . He also founded in his study, among 77 non ulcer dyspeptic patients,50 had H pylori infection(64.9%).[59]

Gwee KA et al (2009) showed when compared studies conducted in western population, Indian studies states that asian group of people have greater relief from non ulcer dyspepsia symptoms after complete eradication of H.pylori.[60]

Abdulla M et al (2009) defines dyspepsia as epigastric pain or discomfort perceived to originate in the upper gastrointestinal tract, including heartburn, acid regurgitation, excessive belching, abdominal bloating, nausea, a perception of abnormal or slow digestion and early satiety.[61]

PEPTIC ULCER DISEASE:

H.pylori is the major cause of peptic ulcer and it usually occurs in middle age to older person.It has been detected in the stomach of more than 90% of gastric ulcer patients who were not in non steroidal anti-inflammatory drug users. Normally peptic ulcer may heal without any active intervention therapy but in presence of H.pylori organism the recurrence of petic ulcer is very common. So active Eradication of H.pylori in these patients facilitates the cure of peptic ulcer and essentially prevents its recurrence. The epidemiology of gastric ulcer is immediately related to that of H.pylori infection, gastritis and gastric

cancer. Studies from various literature in the world shows that prevelance of gastritis is equal to that of H.pylori prevelance.

Kolts BE et al (1993) states that by complete eradication of H.pylori from gastric mucosa helps in curing of peptic ulcer disease and thereby decrease overall prevalence of H.pylori induced peptic ulcer disease .[62]

Vac A(vacuolating cytotoxin) pathologic gene product plays a vital role in the development of peptic ulcer disease .In a study by Zhang C et al (2005) of 286 patient, H.pylori induced gastric ulcer is severe than that of chronic gastritis and also mention that in H.pylori induced gastritis the severity of inflammation is more and there will be diffuse infiltration of inflammatory cells in lamina proria such as neutrophils,eosinophils ,plasma cells which results in severe mucosal inflammation and all this occurs because of H.pylori bacterial products which includes Urease, ammonia ,Vac A protein and other inflammatory mediators such as interleukin-8.[63]

H.pylori produces various enzymes to safe guard itself and to produce the injury to the gastric mucosa. The enzymes includes urease which converts the gastric acidic medium into alkaline medium and thereby make suitable environment for its survival,other enzyme protease which breaks down glycoprotein in the gastric mucus and thereby converts the thick mucus into thin mucus and it also secretes

phospholipase which produce surface epithelial cell injury.In addition H.pylori also release enzyme lipopolysaccharide which recruit inflammatory cells into the gastric mucosa and this inflamed mucosa is more prone for acidic injury. Atlast H.pylori attracted neutrophils secretes myeloperoxides which produces hypochlorous acid and it is converted into monochloramine which cause severe damage to gastric epithelial cells and lamina propria endothelial cells.

Coming to the gross and morphological changes induced by H.pylori in peptic ulcer disease , anterior wall of duodenum is more prone for ulcer than posterior wall. Gastric ulcer is most commonly occur in the lesser curvature of the stomach. Grossly the peptic ulcer is round to oval with sharply punched out lesion with overhanging edges. Histologically it varies from active necrosis to chronic inflammation and atlast result in scarring . Ulcer has 3 zones the outer fibrinoid necrotic layer, middle inflammatory layer predominantly composed of neutrophils and deeper layer shows active granulation tissue which rest on collagenous scar.

According to Konturek SJ et al (2006) mention that complete eradication of H. pylori prevents the increase hydrochloric acid secretion in gastric ulcer patient and helps in healing with prevention of ulcer recurrence and also prevents the H.pylori related carcinoma complication in future.[64]

TUMORS

GASTRIC ADENO CARCINOMA :

The pre neoplastic condition for the development of adenocarcinoma of stomach is Gastric epithelial dysplasia (GED).

The progression from non-neoplastic to carcinoma of stomach involves various steps which includes glandular atrophy,intestinal metaplasia ,dysplasia and atlast into adenocarcinoma. For all of the above mention step the main culprit was H.pylori infection in the gastric mucosa.It plays a vital role in early stage of development of carcinoma. H.pylori associated chronic gastritis leads to severe injury to gastric mucosa which result in increase turnover of epithelial cell to compensate the epithelial degeneration, as a result of this compensatory mechanism incomplete intestinal metaplasia develops due to DNA instability which leads to subsequent development of intestinal type of adenocarcinoma.

Thereby eradication of H.pylori plays an important role in various aspects to prevent the development of carcinoma .It helps in prevention of intestinal metaplasia and atrophy and thereby stops the progression of disease into carcinoma and it also helps in suppression of DNA damage and subsequent proliferation of gastric epithelium.[65]

Patients with younger age group having H.pylori infection were at increased risk for developing carcinoma of stomach compared to older ones.

Infection with strains of H.pylori that carry the cytotoxin associated antigen A (cagA) gene is associated with gastric carcinoma.[66]

H.pylori as designated as class I carcinogen by World health organisation. This is because it produces lifelong proinflammotory response and therby results continous production of free radicals which causes damage to the DNA thereby produces multiple mutations required for the gastric cancer development.

GASTRIC LYMPHOMA:

Gastric lymphoma is strongly associated H.pylori infection and thereby involved in the pathogenesis of low grade lymphoma of Mucosa associated lymphoid tissue (MALT).[67]

Genta et al (1993) reported in young patient about the closest link between the H.pylori infection and gastric lymphoid follicles or 'nodular gastritis'. Microscopically, this nodules were composed of lymphoid follicles in the lamina propria.[68]

The higher prevalence in antral mucosa of lymphoid follicles and aggregates fits well with the distribution of primary gastric lymphomas. The follicles are formed due to H.pylori infection which may leads to gastric lymphoma atlast. In case of H.pylori gastritis the lymphoid aggregates and lymphoid nodules are seen at the base of the antral mucosa.[69]

DETECTION METHODS FOR HELICOBACTER PYLORI :

Diagnostic tests for H.pylori include microbial cultures, histologic or cytologic examination, rapid urease-based tests and serologic studies. Endoscopy dependent invasive methods include rapid urease test (Campylobacter-like organism test)and histology.

Rapid urease test is otherwise known as Campylobacter-like organsism test . It is consider as rapid test for the diagnosis of H.pylori organism . The principle of this test is based on production of urease enzyme by H.pylori . This test is done at the time of doing gastroscopy . The biopsy taken from the antrum is placed in a medium having urea .so the biopsy bearing the H.pylori organism secretes urease enzyme which convert urea into ammonia thereby PH is raised and the colour of the specimen changes from Yellow to Red (positive).

Histologic examination equals or even surpasses culture, especially when positive. However, the patchy nature of the infection requires examination of a minimum of two biopsies: One from the gastric antrum and one from the fundus. The greater the number of biopsies examined, the greater the diagnostic yield, especially in individuals with light infections. Mucosal biopsies have the advantage of allowing one to examine the mucosa for the presence of gastritis or other lesions. Careful examination of four specimens, two from the antrum and two from the corpus, has a high probability of establishing the correct diagnosis of the

infection. The bacteria are generally readily apparent on H&E-stained sections but the infection may be focal and patchy or there may be only sparse organisms, especially if intestinal metaplasia is present.[70]

H.pylori usually detected in gastric antral mucosa and corpus but they are very rare to colonise the cardia. In patient having intestinal metplasia and severe atrophy ,the organism usually identified adjacent non metaplastic and non atrophy areas. They also transform from spiral shape into atypical coccoid forms in unsuitable condition such as usage of proton pump inhibitor or antibiotic etc.,this coccoid form indicates the degenerated forms of H.pylori and they are avirulent.[71]A number of special stains aid in H.pylori detection including Dieterle silver, Warthin-Starry, Gram, toluidine blue, Giemsa, Wright-Giemsa, Brown-Hopps, acridine orange, or Diff-Quik stains. In addition, there are good immunohistochemical reagents that detect the organism. These are particularly useful in detecting the coccoid forms of the bacteria.

One can also identify H.pylori in routinely prepared cytology specimens. Gastric mucosal brushings sample larger surface areas than a biopsy, thus serving as a useful adjunctive diagnostic test. H.pylori bacteria are easily detected microscopically in smears stained with H&E, (modified) Giemsa, Papanicolaou and silver-based stains (Warthin-Starry and Steiner). The availability of an anti-H.pylori polyclonal antibody allows immunohistochemical identification. Other procedures have been

described such as, determination of gastric juice ammonia and reverse transcriptase- polymerase chain reaction (RT- PCR) amplification as well as methods for identifying specific genotypes employing random amplified polymorphic DNA analysis (DNA fingerprinting).

Since culture, cytologic and histologic examination and rapid urease-based examinations all require endoscopy, less expensive and noninvasive diagnostic tests have been developed; the most popular is the urea breath test. They are indicated for the initial diagnosis of the infection and to follow patients for infection eradication. In this test patient is ask to swallow the urea labelled with radioactive isotope,the result of this test shows detection of isotope labelled carbondioxide in the exhaled breath which indicates that urea was split into ammonia by the enzyme urease which is produced in abundant by H.pylori organism present in the gastric mucosa. This test proves presence of H.pylori infection.

H.pylori bacteria elicit antibody responses, allowing serologic testing. Serum enzyme-linked immunosorbent assays (ELISA) tests detect H.pylori antibodies and indicate current or past infection. The test has a sensitivity of 80% to 100% and a specificity of 75% to 100%.There may be variations in the sensitivity based on strain differences. Serologic testing is not useful for detecting infection elimination. Stool antigen tests can be used to follow patients to determine if the infection has been

eradicated if used after 8 weeks following treatment. They are particularly useful in children.Other noninvasive methods includes assays for exhaled C and C- labeled urea.[72]

At last coming to the Immunohistochemistry(IHC),it is an time consuming and very costly procedure approximately takes one to twenty four hours but sensitivity and specificity is very high because H.pylori can be easily identified in the slides. Immunohistochemistry involves two disciplines – immunology and histology.Immunohistochemistry is used to determine expression of particular antigen and its micro anatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. These differences can specifically identify the lineage of cell population and define biologically distinct populations of cells within the same lineage. In Direct labelling method Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of multiple antigens which require separate incubation with respective antibodies. In Indirect labelling method enzymes are labelled with the secondary antibody, which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versality, higher working dilution of primary antibody, secondary antibodies against primary antibodies of different species and easy to prepare.[73]

ESOPHAGEAL LESIONS:

ESOPHAGITIS:

Esophagitis has many causes, the most common being gastroesophageal reflux, infections and drugs. Esophageal biopsies are taken to determine the etiology of the esophagitis, to assess the consequences of the inflammation, to follow the course of the underlying disease and to gauge therapeutic responses.

Esophageal inflammation can be acute, chronic, or mixed. Mild esophageal injury results in reversible mucosal changes and transient inflammation. Changes associated with acute damage include the presence of balloon cells and inflammatory cells (particularly mononuclear cells) and eosinophils. Basal cell hyperplasia and papillary elongation develop and vascular lakes form. In severe esophagitis, ulcers, erosions, or neutrophils may be seen. Chronic damage leads to submucosal fibrosis or strictures. Patients with longstanding reflux esophagitis may develop Barrett esophagus.

RELATIONSHIP OF REFLUX ESOPHAGITIS TO HELICOBACTER PYLORI INFECTIONS:

Reflux Esophagitis:

The term gastroesophageal reflux (GER) refers to the retrograde flow of gastric and sometimes duodenal contents into the esophagus.

Studies addressing the relationship of H.Pylori infection to GERD often reach conflicting conclusions. This results from the fact that the interplay of H.pylori infections and GERD is complex and complicated by the common use of proton pump inhibitor (PPI) therapy in these patients. At the heart of the debate is the link between gastric acid secretion, H.pylori infection and GERD. In patients with gastric ulcer and corpus gastritis, the impact of H.pylori infection varies substantially producing wide variation in patterns of acid secretion. Bacterial eradication helps in contolling the H.pylori induced gastritis and its complication but in case of GERD it plays a controversial role because eradication of organism results in increase hydrochloric acid secretion and thereby increase the aggressiveness of refluxed gastric juice to the esophageal mucosa. In contrast, duodenal ulcer patients typically have antrum-predominant H.pylori gastritis and a well-preserved acid-secreting mucosa. In these patients, H.pylori infections may make the acid-secretory mechanism hyperresponsive to stimulation, increasing acid production. In this patient group, H.pylori infections can increase the aggressiveness of the gastric juice to the esophageal mucosa.so thereby eradication of H.pylori results increase in GERD prevalence.[74]

Approximately one third of patients with chronic GERD symptoms are endoscopically normal. Low-grade esophagitis is only evident histopathologically. Areas of patchy erythema and red streaks are the first

endoscopic abnormalities. Later erosions and ulcers develop; these predominate distally and taper off proximally. The esophagus appears friable, diffusely reddened and hemorrhagic it bleeds easily. As the disease progresses, the ulcers become confluent, even circumferential. Strictures or Barrett's esophagus characterize severe chronic disease. Prolonged reflux may result in esophageal shortening. Inflammatory polyps may be present. The distinction between the squamous and the columnar epithelium becomes less clear. Several endoscopic classifications have been developed to evaluate the esophageal mucosa.[75]

HISTOLOGIC FEATURES

Biopsies are performed to confirm the diagnosis of GERD to document complications, including esophagitis, BE or tumor development; and to rule out the presence of coexisting infections.

Repetitive episodes of tissue injury and healing produce histologic features that reflect disease activity at the time of examination, superimposed on changes from previous injurious episodes. Biopsies from patients with heartburn commonly show only basal cell hyperplasia without inflammation. The basal hyperplasia can progress to frank esophagitis. There are four stages of reflux esophagitis: (a) acute (necrosis, inflammation and granulation tissue formation); (b) repair (basal cell hyperplasia and elongation of the papillae); (c) chronic

(fibrosis and formation of Barrett esophagus); and (d) complications (dysplasia and adenocarcinoma).

BARRETT'S ESOPHAGUS(BE):

Barrett's esophagus(BE)is an acquired metaplastic change that results from longstanding GERD. It results from a combination of substances in the refluxate including acid, bile salts, lysophospholipids and activated pancreatic enzymes. In this abnormal milieu, multipotential immature stem cells differentiate into various epithelial types, including columnar epithelium, which is more resistant to acidic digestion and which is able to regenerate more rapidly than the native squamous epithelium . Once established, Barrett's esophagus is a highly proliferative mucosa.

The development of Barrett's esophagus is a multistep process with at least three distinct phases. During the initiation phase, genetically predisposed patient suffering from GERD develop reflux esophagitis. This leads to the formation of a metaplastic epithelium with features of intestinal columnar epithelium. The metaplastic columnar cells of Barrett's esophagus could derive from three sources: (a) metaplasia of squamous epithelium, similar to that seen with vaginal mucinosis; (b) from the mixed squamous/columnar cell population at the transitional zone, as seen in cervical metaplasia; or (c) from the columnar cells of the esophageal glands, such as may be associated with ulcer repair.

Circulating bone marrow derived stem cells (BDSCs) have been proposed to be the source of metaplastic cells in the stomach in response to H.pylori gastritis. Recruitment of BDSCs in response to reflux-induced inflammation might serve as another potential source of Barrett's esophagus.

During the formation stage, the metaplastic epithelium exposed continuously refluxed acid content will results increase in metaplastic changes . A long and multifaceted progression phase follows, during this phase metaplastic epithelium progress into dysplasia and the end results in adenocarcnioma. [76]

GROSS AND ENDOSCOPIC FEATURES:

Barrett's esophagus appears beefy red and velvety, contrasting with the lighter pink-tan colored, smooth squamous mucosa. The Squamo Coloumnar Junction(SGJ) often lies within 30 cm of the incisor teeth and often coexists with a hiatal hernia, strictures, diffuse esophagitis, or esophageal ulcers. Grossly, there are several distinct patterns of Barrett's esophagus: Circumferential, islands and finger like projections or tongues. The island type accompanies less severe epithelial injury than the circumferential type and probably represents an earlier stage, which then progresses to the circumferential lesion. Sometimes it is difficult to distinguish the distal border of the metaplastic epithelium from the adjacent gastric mucosa with which it may appear to merge. Locating the

gastric folds helps delineate the beginning of the stomach. Patients with Short Segment Barrett Esophagus (SSBE) have short tongues or patches of red mucosa lying <2 cm above the gatroesophageal junction(GEJ).

Since it may be difficult to endoscopically distinguish areas of intestinal metaplasia from other columnar epithelium, various additional endoscopic approaches may be used to evaluate the mucosa. These include magnification endoscopy, endoscopic optical coherence tomography, chromoendoscopy, endoscopic confocal imaging, light-scattering spectroscopy and in vivo fluorescence endomicroscopy. These advanced imaging methods may enable the endoscopist to detect intestinal metaplasia in a background of gastric epithelium; to detect foci of dysplasia and early neoplasia in a background of intestinal metaplasia; and to distinguish early invasive carcinoma from mucosal dysplasia [77]

Typically the endoscopist biopsies the following areas: The stomach just distal to the upper end of the gastric folds, particularly along the lesser curvature; 1 to 2 cm above the GEJ; tongues of mucosa or irregular areas above the SCJ; and the SCJ and squamous epithelium of the native esophagus. Biopsies at the upper end of the gastric folds may allow one to determine whether there is gastritis, particularly H.pylori induced gastritis and possibly intestinal metaplasia. This biopsy may be within a hiatal hernia. These biopsies can detect localized carditis,

localized intestinal metaplasia, reactive changes, acute inflammation and possibly eosinophils in the squamous mucosa.

HISTOLOGY OF BARRETT'S ESOPHAGUS

There are two major problems in the pathologic evaluation of patients with Barrett's esophagus: Overdiagnosis of Barrett's esophagus and overdiagnosis of dysplasia in the setting of Barrett's esophagus. The histology of the columnar-lined esophagus displays heterogeneous histologic features with respect to the types of glandular mucosa that are present and the surface architecture. The definition of Barrett's esophagus requires histologic confirmation of intestinal metaplasia in biopsies taken from the columnar regions of the esophagus. The metaplastic Barrett's esophagus epithelium resembles either small intestinal absorptive cells (complete intestinal metaplasia) or incomplete intestinal metaplasia (resembling colonic epithelium). In the latter, the cells lack a distinct brush border and the associated enzymes that normally characterize small intestinal absorptive cells. There is debate over whether incomplete metaplasia poses a higher risk than the complete type, but since both types confer a neoplastic risk, subtyping is not indicated. If any goblet cells are seen, one can make the diagnosis of Barrett's esophagus when the biopsy derives from the esophagus .

Examination of multiple biopsies and multiple levels helps identify this patchy process. The epithelium covering the mucosal surface and pits

commonly contains a mixture of gastric foveolar cells and intestinal cells. The latter include goblet cells, intestinal columnar cells, endocrine cells (containing serotonin, somatostatin, calcitonin, pancreatic polypeptide and secretin) and sometimes Paneth cells. The majority of the intestinal columnar cells are so-called intermediate, principal, or pseudoabsorptive cells that have characteristics of both absorptive and secretory cells. A villiform architecture may be present on the surface. H. pylori may be found in the esophagus of some patients with Barrett's esophagus but only when it is also present in the stomach. It may contribute to the severity of the inflammation seen in Barrett's esophagus.

Goblet cells are usually readily identifiable in H&E-stained sections by their round supranuclear mucin accumulation. While goblet cells contain acidic mucin that stains intensely blue with Alcian blue staining at pH 2.5, routine Alcian blue staining is usually not necessary. Alcian blue positive cells are also found in normal esophageal submucosal glands and their ducts. These submucosal glands are readily distinguished from Barrett's esophagus because of their rounded, grouped lobular configuration and their resemblance to minor salivary glands as well as by their diffuse positivity for Alcian blue at pH 2.5. The entire esophageal glandular lobules stain intensely contrasting with the individually scattered intensely positive goblet cells typical of Barrett's esophagus.

Careful histologic attention should be paid to potential Barrett's esophagusmimics, particularly pseudogoblet cells. These columnar cells are hyperdistended gastric foveolar cells. They contain a mucinous droplet that is larger than the typical foveolar cell but smaller than the usual goblet cell. They occur in the surface epithelium at the GEJ and distal esophagus. They may occur in the presence or absence of true goblet cells. The cells stain positively with Alcian blue at a pH 2.5; for this reason they are sometimes referred to as the columnar blue. However, the pseudogoblet cells stain less intensely than true goblet cells. If only Alcian blue positive columnar cells are present in the absence of true goblet cells, the diagnosis of Barrett's esophagus should not be made. Because of the lack of specificity of Alcian blue staining for true goblet cells, there has been interest in finding a more specific marker of intestinal goblet cells. Markers of interest have included stains for sulphomucins and sialomucins. Another promising marker is MUC2, which may be specific for intestinal metaplasia in Barrett's esophagus .[78]

Of note, the muscularis mucosae is hyperplastic in the distal esophagus and in some areas collagen-rich fibrous tissue replaces the muscularis mucosae. An understanding of these features is important in two situations: The first is the correct interpretation of alterations that may affect the submucosal glands and the second is the correct staging of invasive malignancies . The fibroblastic or muscular abnormalities may

deform the ducts of the submucosal glands, causing the glands to dilate. The combination of irregular ductal compression in the presence of atypical epithelial cells lying in the fibrous tissue can cause difficulty in differentiating normal or dysplastic esophageal glands trapped in the collagen-rich fibrotic tissue from invasive cancer .[79]

MATERIALS AND METHODS

This study was proposed and conducted in the Tirunelveli Medical College Hospital. A pilot study was done and approval of the ethical committee of the Tirunelveli Medical College & Hospital was obtained.

Patients presented to the outpatient department of the Department of Medical Gastroenterology at Tirunelveli Medical College Hospital and a private Medical Gastroenterology Clinic, during the period - January 2010 to September 2012. A total of 145 cases presenting with features of acid peptic disease and GERD were examined, in that 100 patients were selected for our study based on a set of inclusion and exclusion criteria. Exclusion criteria includes patients with acute gastritis,complications like bleeding and patient with gastric malignancy/esophageal malignancy.

Endoscopy was carried out using Olympus GIFSQ 30 video endoscopy system in the Department of Medical Gastroentrology at the Tirunelveli Medical College and Hospital. Endoscopy finding was recorded using a standard proforma . Biopsy samples was taken from sites of antrum of stomach .Concurrent examination of the esophagus was done in all these cases and biopsies were taken from them.We received biopsy of the esopahgus from 76 cases.

The specimens were immediately fixed in 10% formalin and processed routinely. Four microns sections were taken from both the tissues and stained with hematoxylin and eosin. Sections were also

stained with special stain using carbol fuchsin and light green counterstain for detection of Helicobacter pylori and with the conventional Warthin –Starry silver stain for detection of Helicobacter pylori.

Each section was analysed for the presence of epithelial changes which includes surface irregularity, epithelial pits ,drop out necrosis and microerosion. The histological changes such as inflammation,mucosal atrophy ,neutrophilic activity , intestinal metaplasia and H.pylori status was graded according to the Sydney system. The results and observations were tabulated and analysed for their significance. An attempt was made to study the efficiency of a stain for demonstrating helicobacter pylori in gastric biopsies using dilute carbol fuchsin light green stain and comparison were made with that of warthin starry stain.

HAEMATOXYLIN AND EOSIN (H&E):

Procedure

1. Dewax the section and hydrate through graded alcohols to water.
2. Remove fixation pigments if needed.
3. Stain with Harris Haematoxylin for 2-3 minutes.
4. Wash in running tap water .
5. Differentiate in 1% Acid Alcohol(1 per cent HCL in 70 percent alcohol) for 5-10 sec.
6. Wash and blue with running tap water until the section turns blue, followed by tap water wash.
7. Counter stain with aqueous eosin for 2 minutes.
8. Dehydrate with absolute alcohol (2-3changes).
9. Clear with 2-3 changes of xylene.
10. Mount using Dibutyl phthalate Polystyrene Xylene (DPX).

Results :

Nuclei	:	Blue
Cytoplasm	:	Varying shades of pink
Muscle fibers	:	Deep pink/red
Red blood cells	:	Orange/red
Fibrin	:	Deep pink
Organism	:	Pink

CARBOL FUCHSIN AND LIGHT GREEN STAIN :

Specimens :

For fixation of the gastric biopsies Formalin is used

Controls :

H.pylori positive tissue were used as controls all the times .

Staining solutions:

Working solution: For carbol fuchsin

- Carbol fuchsin-20ml
- Distilled water-80ml

Light green counterstain in Gallegos (Brown and Hopps 1972)differentiating solution:

- Distilled water-97ml
- 40%formaldehyde-2ml
- Glacial acetic acid-1ml
- Add 20 drops of 2% light green

Twenty drops of 2% light green per 100ml of Gallegos solution is recommended for a pale counterstain that should not mask any organisms, but can be adjusted to suit the users preferences.

Light green 2% in 1% acetic acid

- Distilled water -99ml
- Glacial acetic acid-1ml
- Light green-2g

Acetic acid 0.1% in 90% ethanol

- Absolute ethanol-90ml
- 1%aqueous acetic acid-10ml

Staining procedure:

1. Dewax in Xylene, hydrate through graded alcohol and bring the section to water.
2. Rinse in buffered distilled water (pH 6.8).
3. Stain with dilute carbol fuchsin 5 -10min.
4. Wash the section in running tap water.
5. Rinse with absolute ethanol for 10 seconds until the red colour disappear.
6. Again Wash the section in running tap water.
7. Dip the section in light green for 1 minute which acts as counterstain.
8. Rinse briefly with 0.1% acetic acid in 90% ethanol. This rinse after differentiation assists in removing backround staining and initiates dehyhydration.
9. Dehydrate rapidly in absolute ethanol.
10. Clear in two or three changes of xylene.
11. Mount in DPX.

Results :

- H.pylori - Takes Bright red
- Gastring lining - Pale green
- Nuclei and other cell structures - Shades of pink to red

WARTHIN –STARRY SILVER STAIN

PROCEDURE

Sections

Formalin-fixed , paraffin sections.

Solutions

- Acetate buffer,PH 3.6
- Sodium acetate : 4.1 g
- Acetic acid : 6.25ml
- Distilled water : 500ml

1%silver nitrate in pH 3.6 acetate buffer.

Developer 3 g of hydroquinone in 10ml pH 3.6 buffer and mix 1ml of this solution and 15ml of warmed 5% scotch glue or gelatine; keep at 40 degree celcius. Take 3 ml of 2% silver nitrate in pH 3.6 buffer solution and keep at 55 degree celcius mix these two solutions immediately before use.

Method

1. Deparaffinize and rehydrate through graded alcohols to distilled water.
2. Celloidinize in 0.5% celloidin,drain and harden in distilled water-1min.
3. Impregnate in pre-heated 55-60 degree celcius silver solution(b),90-105 minutes.
4. Prepare and preheat developer in a water bath.

5. Treat with developer(solution c) for $3^{1/2}$ minutes at 55 degree celcius. Section should be golden-brown at this point .
6. Remove from developer and rinse in tap water for several minutes at 55-60 degree celcius, then buffer at room temperature.
7. Tone in 0.2% gold chloride .
8. Dehydrate, clear and mount .

Results

- Spirochetes : Black
- Background : Golden yellow

OBSERVATION AND RESULTS

TABLE :I

Age and gender distribution of 100 patients presented with gastritis

Age	No. Of patient	No. Of Male patient	No.Of Female patient
11-20	5	3	2
21-30	20	16	4
31-40	26	17	8
41-50	18	9	10
51-60	9	7	2
61-70	12	8	4
>70	10	10	0
Total	100	69	31

In hundred cases of gastric biopsies studied, sixty nine cases (69%) were males and thirty one cases (31%) were females with a male to female ratio of 2.22:1. Of the 100 patients studied, the age ranging from 11 to 84 years (Table 1) with a mean age of 35.54 years. Peak incidence of occurrence of gastric lesions were third and fourth decade with twenty cases(20%) and twenty six cases(26%) respectively.

CHART 1:

Age and gender distribution of 100 patients presented with gastritis

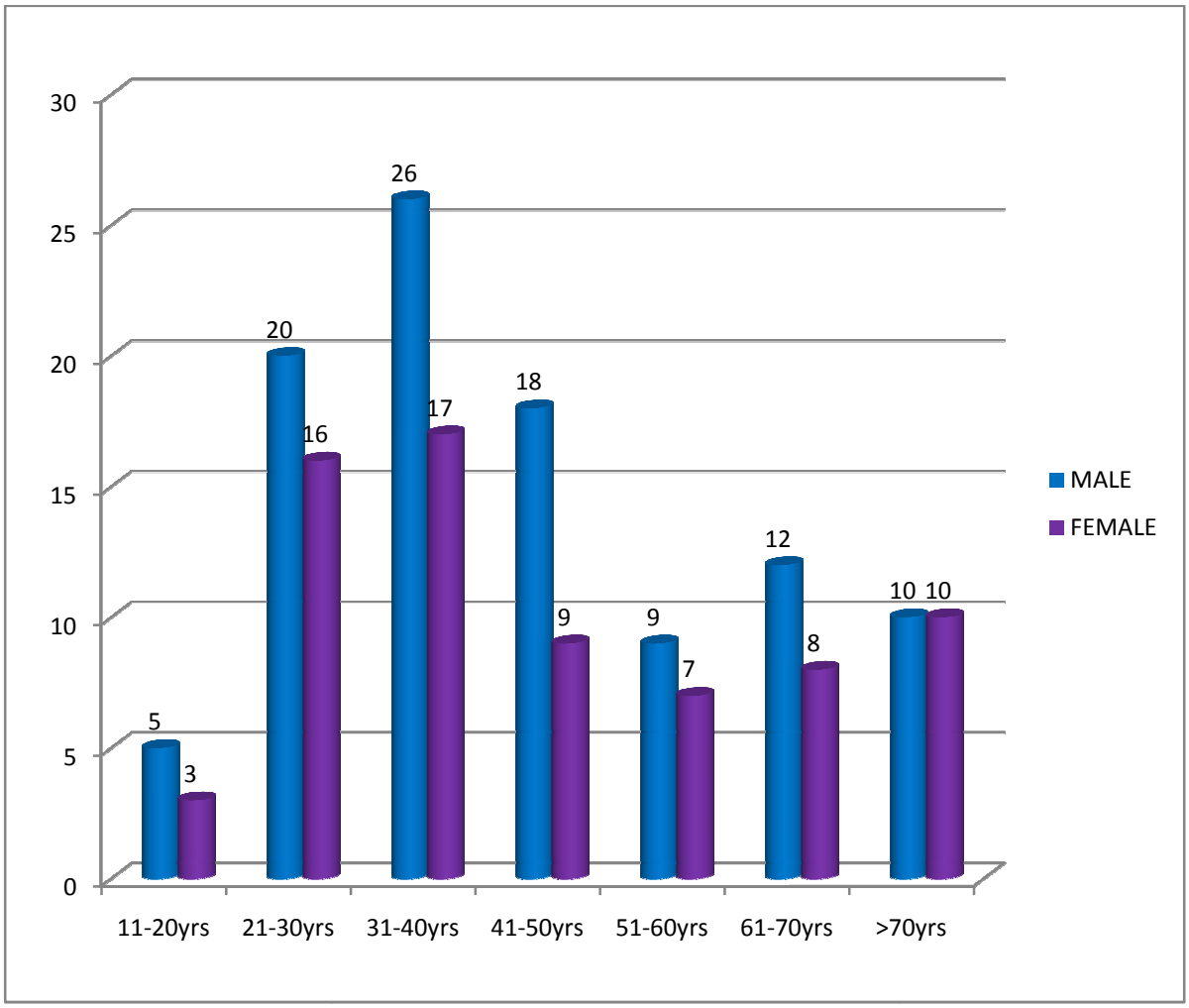


TABLE II:

Clinical symptoms for patient with gastritis

Clinical symptoms	Number of patient with symptoms
Nausea	78
Vomiting	67
Abdominal pain	89
Heart burn	92

Patient with chronic gastritis mostly presented with following symptoms such as nausea, vomiting, abdominal pain and heart burn.Among the clinical features in this present study most common was heart burn followed by pain abdomen and nausea and most of the patient presented with more than one symptoms in our study [Table II].

TABLE:III

Helicobacter pylori status in various age groups

Age	No. Of patient	H.pylori positive	H.pylori negative
11-20	5	1	4
21-30	20	10	10
31-40	26	18	8
41-50	18	14	4
51-60	9	7	2
61-70	12	8	4
>70	10	6	4
Total	100	64	36

The age distribution of H.pylori shows a peak incidence at fourth to fifth decade patient . Predominantly fourth decade patient shows peak incidence of H.pylori infection.In fourth decade patient among 26 patient 18 patient were found to be positive for H.pylori infection in our positivity. After that the incidence of H.pylori infection is decreased with increase in age (Table III).

CHART 2:

HELICOBACTER PYLORI STATUS IN VARIOUS AGE GROUPS

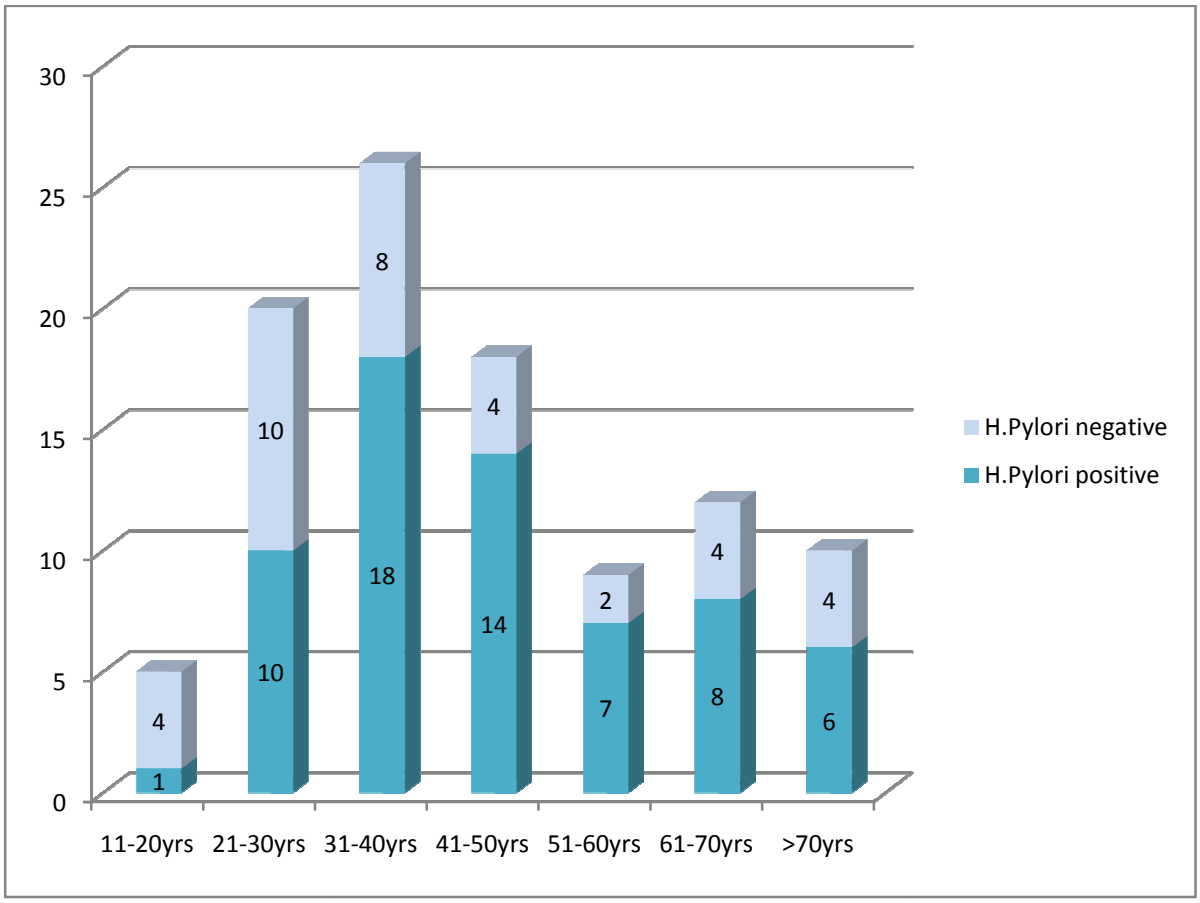


TABLE IV:

Helicobacter pylori status in gender

Sex	No . of patient	H.pylori positive	H.pylori negative
Male	69	44	25
Female	31	20	11
Total	100	64	36

In our study, among the sixty four cases of Helicobacter pylori associated gastritis , fourty four (68.75%) were males and twenty (31.5%) were females with a ratio of 2.2:1. This shows male predominance for H.pylori infection (Table IV).

CHART 3:

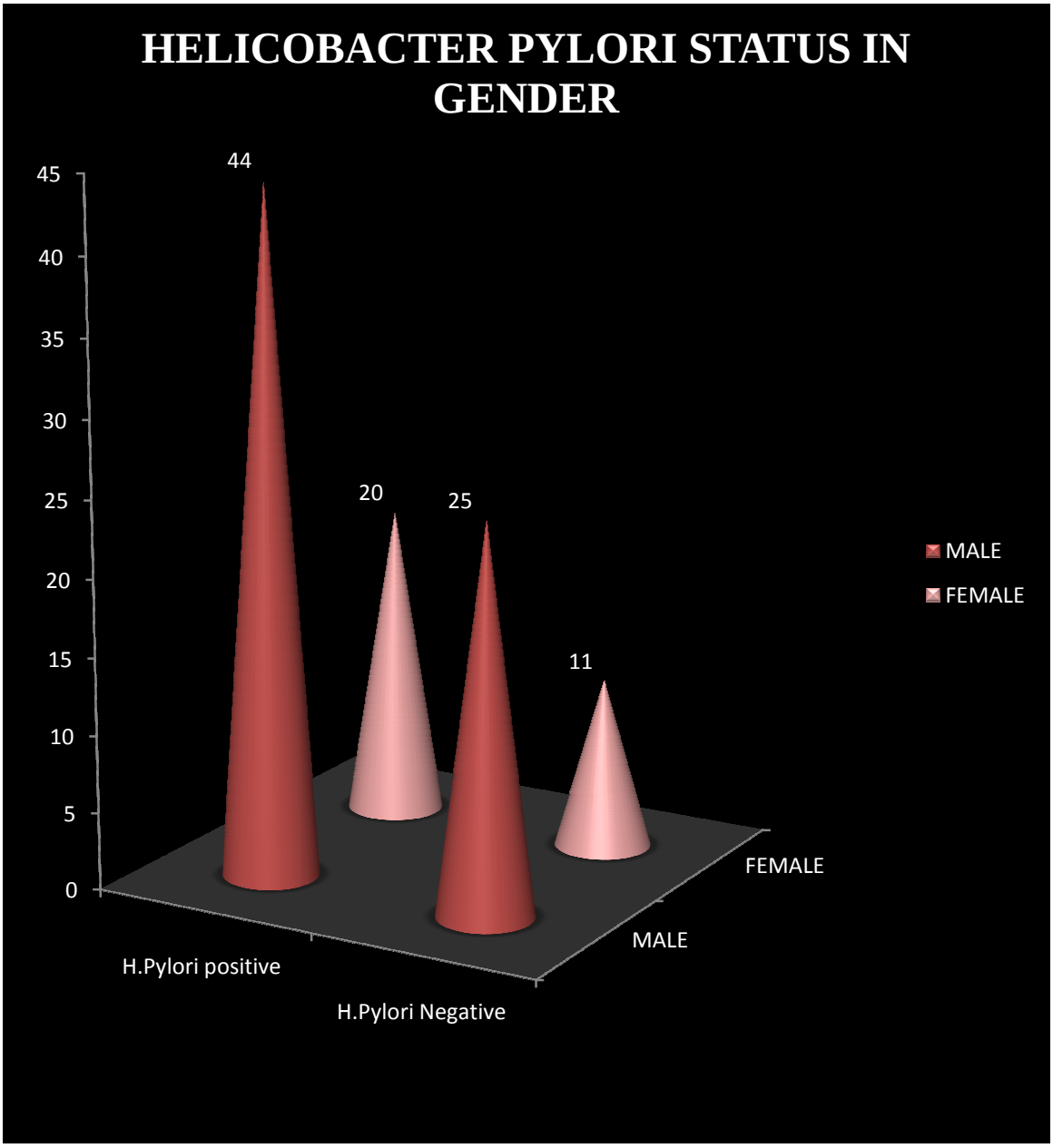

HELICOBACTER PYLORI STATUS IN GENDER
45
40
35
30
25
20
15
10
5
0
44
20
25
11
MALE
FEMALE
FEMALE
MALE
H.Pylori positive
H.Pylori Negative

TABLE V:

Comparison of H.pylori status with that of inflammation, activity, atrophy and intestinal metaplasia

Mucosal changes	Total no. of cases	H.pylori positivity	Percentage
Inflammation			
1)Mild	27	9	33.3%
2)Moderate	47	41	87.23%
3)Severe	15	14	93.33%
Activity	65	55	84.6%
Atrophy	33	23	69.6%
Intestinal metaplasia	22	16	72.7%

In our present study number of antral biopsies with significant inflammation ,activity,intestinal metaplasia and atrophy were analysed and graded according to the Modified Sydney system. We had 27 cases with mild inflammation ,47 with moderate inflammation and 15 cases with severe inflammation .Correlating the H pylori positivity status with that of the type of inflammation,we had 33.3% positivity in mild inflammation,87.23% positivity in moderate inflammation and 93.33 % positivity in cases with severe inflammation. This observation is

statistically significant. The positivity rate increases with that of the density of the bacteria.

Accounting the activity status in the 65 cases of our study 55 cases [84.60%] showed positivity for H.pylori. Among 22 cases with intestinal metaplasia in our present 72.7% [16 cases] were shown positivity for H.pylori organism.(Fig 1-17)

CHART 4:

Association between Inflammation and H.pylori infection

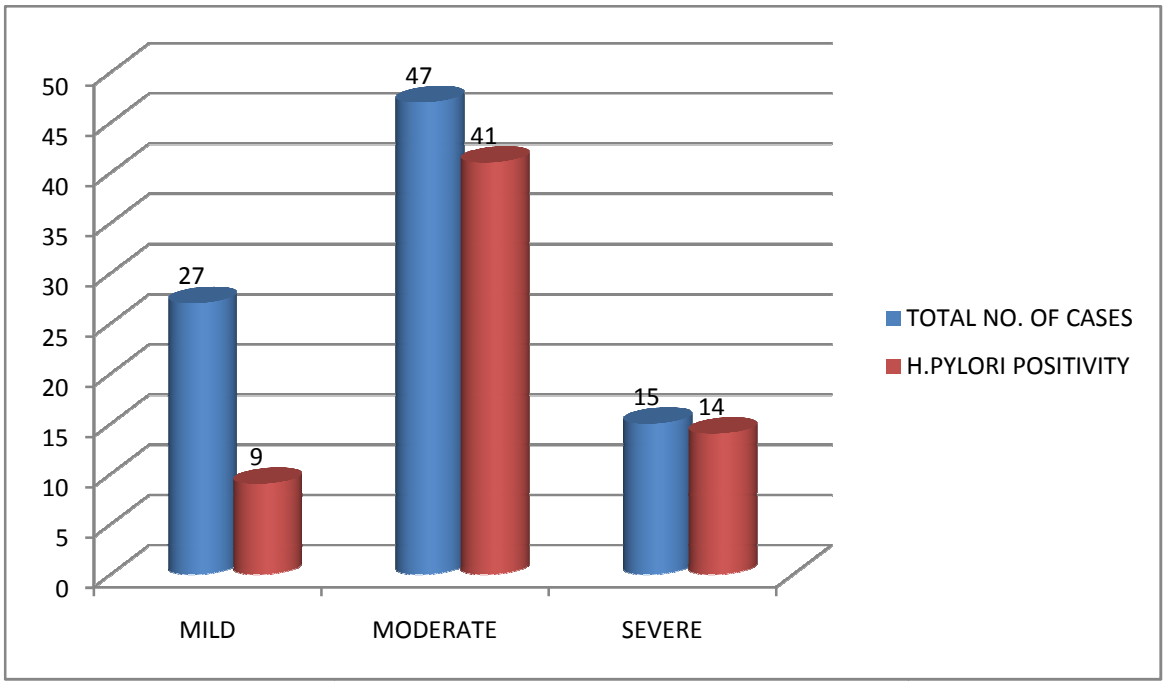


CHART 5:

Association of H.pylori infection with activity,atrophy and intestinal metaplasia

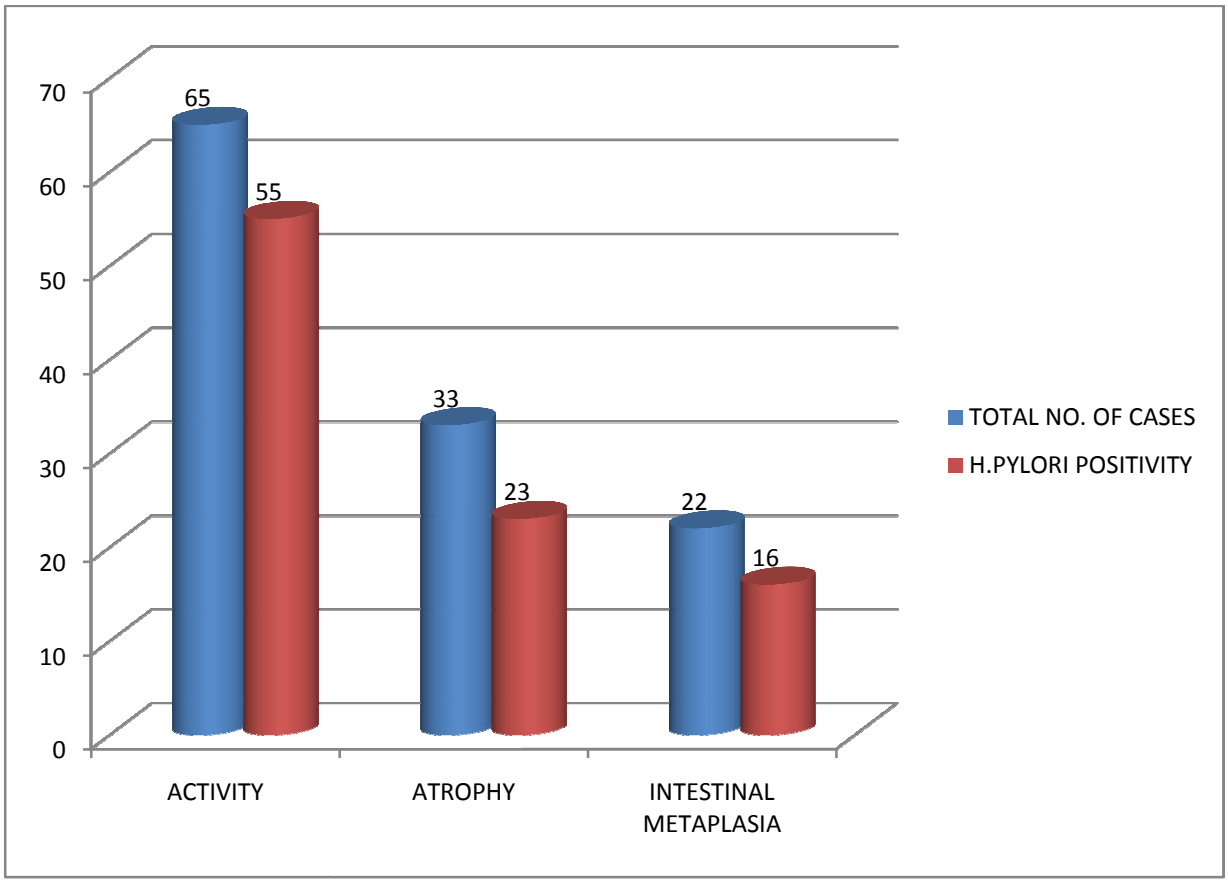


TABLE VI:

Histomorphological Changes in H pylori Positive Chronic Gastritis

Nature of Lesion	No of cases
Mucosal Hyperemia	50
Mucosal edema	52
Surface epithelial degeneration	49
Focal epithelial regeneration	38
Surface erosion	54
Foveolar hyperplasia	38
Lymphoid aggregates	56
Lymphoid follicles	50
Eosinophilic infiltration	12
Intestinal Metaplasia	16
Atrophy	33
Total cases	64

TABLE VII:

Comparison of H.pylori positivity with light green carbol fuchsin stain and Warthin Starry stain

H.pylori status	Light green carbol fuchsin	Warthin starry stain
H.pylori positivity	61	57
H.pylori negative	39	43

In our present study apart from conventional H&E stain to detect H.pylori ,we made a attempt to detect the H.pylori organism using Light green stain and compare the staining qualities with that of Warthin – Starry Silver stain .We found that in 61 cases we were able to detect the organism using Light green carbol fuchsin and in 57 cases using Warthin –Starry stain. Both the stains were positive for 56 cases.(Fig 18-24)

CHART 6:

Comparison of carbol fuchsin light green and warthin starry stain

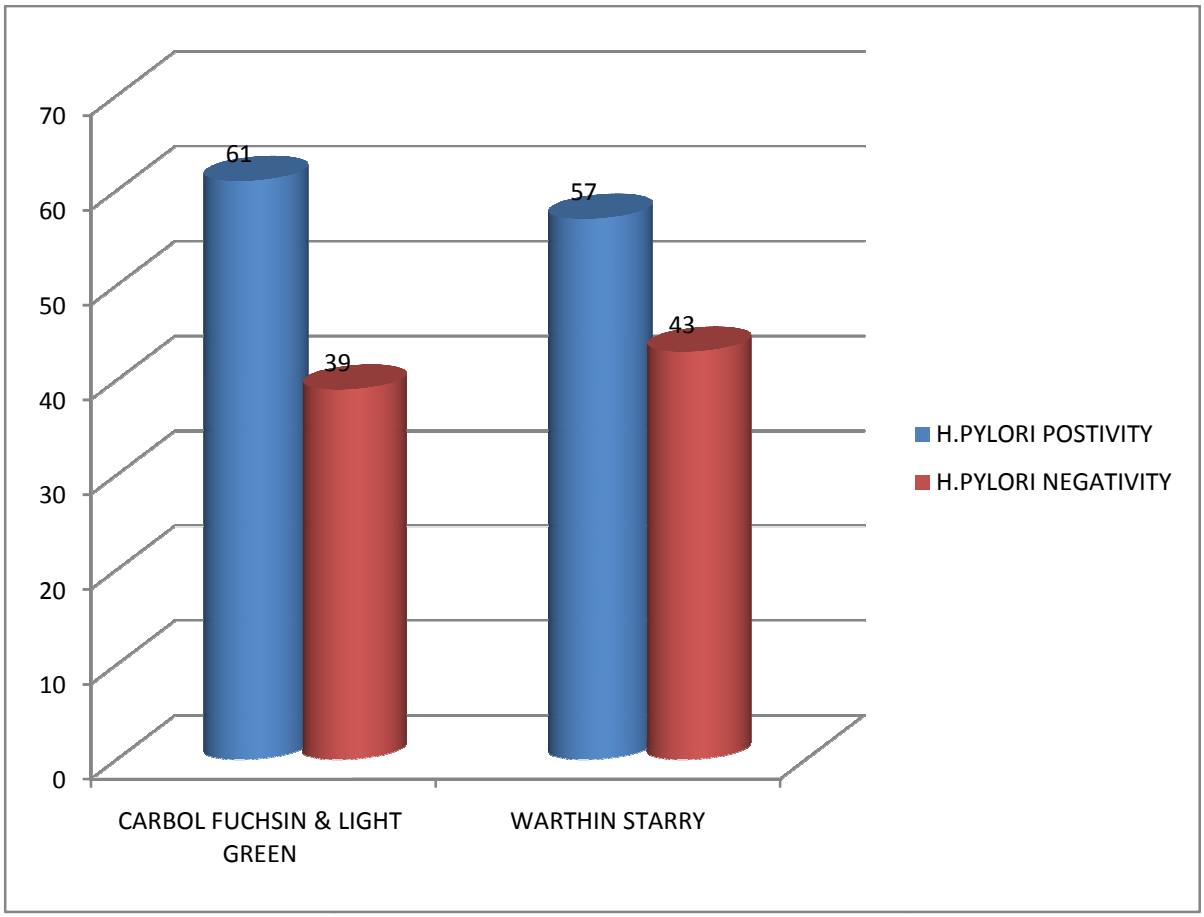


TABLE VIII:

Male and female distribution of positive & negative cases of Helicobacter pylori in warthin and a stain using carbol fuchsin and light green as counter stain:

Method	Warthin starry stain		Carbol fuchsin and light green stain	
	POSITIVE	**NEGATIVE**	**POSITIVE**	**NEGATIVE**
Male	41	28	43	26
Female	16	15	18	13
Total number of cases	57	43	61	39

TABLE IX:

Comparison of various factors between light green carbol fushin and warthin starry stain :

Special stain	Relative cost	Average time	Reproducibility of technique
Warthin starry	Expensive	2hrs	Relatively Good
Carbol fuchsin light green	Cheaper	10-15min	Good

In our study we also made comparison about the relative cost,average time and reproducibility of technique between Warthin starry and carbol fuchsin light green . In that we found that Light green carbol fuchsin is better than that of Warthin starry stain as it is cheaper, less time consuming and detection of the bacteria is easier (pink spiral forms against green background) and for easy reproducibility of technique.The problem we encountered with Light Green was in cases with less number of bacteria, in which Warthin- Starry stain was much better.

TABLE X:

Distribution of various esophageal lesion:

Distribution of esophageal lesion	Number of cases
Chronic reflex esophagitis	28
Chronic non specific esophagitis	18
Vascular ectopia	10
Barrets esophagus	14
Normal histology	06
Total cases	76

Endoscopic appearance of the esophagus was also studied in the process of evaluating the cause for the acid peptic disease and GERD.Biopsies were taken from the lesions as and when necessary.

We had esophageal biopsies taken from 76 cases out of the total 100 cases. In that 6 of the cases the esophagus did not show any remarkable pathological changes.

The most common esophageal lesion seen in our series of patients was that of Chronic Reflux Esophagitis 28 cases (36.8%), followed by chronic non specific esophagitis with 18 cases .The next significant esophageal pathology noted was that of Barrett's esophagus in 14 of our cases (18.42%) followed by vascular ectasia of esophagus 10 cases. (Fig 25,26)

DISCUSSION

The present study was conducted on 145 cases of patients presenting with symptoms of acid peptic disease to the outpatient clinic of Medical gastroenterology and a private Gastroenterology clinic at Tirunelveli.The endoscopy study was done on 100 cases only and necessary biopsies were taken from them for the study.

The overall positivity of H pylori in our study was 64% (64 cases out of 100 cases) (Table IV). This is close to the observations of Vijaya VA et al (1999)[80] who found a positivity of 61.4% in her series, Abbas et al (2001)[81] found a positivity of 62% in his study on Pakistani population,Manes et al (1999)[82] in a case control study carried out in Italy found a positivity of 62% .However Misra et al (2001)[83] had a higher positivity of 71% in his series of cases studied for the prevalence of H.pylori in gastric antral biopsies.

The study population comprised of patients with age ranging from 11 to 84 (Mean age was 35.54%).The maximum number of cases were belonging to 4 th decade (26%),followed by 3 rd decade with 20 cases (Table I).This is akin with the observations of Abbas et al (2001)[81] who had a mean age of 45 years & Csendes et al (1997)[84]with a mean age of 43 years.

Correlating the H pylori positivity with the age of the individuals, there is a gradual increase in the incidence of H pylori status with the age

of the individuals.The positivity is 50% in the age group of 21-30 years,and it gradually ascends to 69% in the age group of 31-40 years with much higher percentage of 77.7% seen in age group of 41- 60 years. There is a slight fall in the positivity rate in individuals more than 60 years. The percentage of positivity is 66.6% and 60% respectively (Table III) .This is similar to the observations of Vijaya VA (1999)[80] who found a peak positivity rate of 67.2% in age group of 31-40 years followed by 63.8% in 50 to 59 years of age.She had also reported a fall in the positivity rate after 60 years .Our observations of fall in positivity correlates well with this study.In her study she had observed a very high percentage of 69.3% in patients belonging to age group of 20 to 29 years which we did not find in our series. We had a positivity of only 50 % in those cases.

Regarding the gender distribution of cases , men out numbered women in our series close to the observations of literature. We had 69 male cases and 31 female cases. The M: F ratio being 2.2 :1(Table I).This is similar to the observations made by Niv et al (1996)[85] who found a ratio of 1.6:1 in their series.Correlating the positivity status to the gender of the study group, H pylori positivity was found in 63.7 % of men and 64.5 % women with a mean positivy of 64 %.This observation also correlates well with that of Vijaya VA et al (1999) loc.cit[80] who found a positivity of 63.1% in the men and 56.5% in women.

The number of antral biopsies with significant inflammation was analysed and grade according to the Modified Sydney system.We had 27 cases in mild inflammation,47 cases with moderate inflammation and 15 cases with severe inflammation.Correlating the H pylori positivity status with that of the type of inflammation,we had 33.3% positivity in mild inflammation,87.23% positivity in moderate inflammation and 93.33 % positivity in cases with severe inflammation(Table V).This correlates well with the study of Vijaya VA et al loc.cit (1999)[80] who found a positivity of 57% in cases with mild inflammation,88.4% in cases with moderate inflammation and 100% in cases with severe inflammation.It thus justifies the significant association of presence of inflammation and the H pylori positivity.We had a lesser percentage of positive cases with severe inflammation when compared with the study cited above.Higher positivity was observed in cases with moderate inflammation in our study.Another significant observation made in the present study was greater density of organisms was found with higher grade of inflammation.In mild inflammation the presence of inflammatory cells were restricted to the superficial mucosa at the level of the pits, neck of pyloric glands and the lamina propria whereas in severe inflammation the cells infiltrated deep into the muscularis mucosae.

Regarding activity status we had 65 cases with activity according to the Sydney scoring system.Analysis of the H pylori status in these cases showed a positivity of 84.6% (55 cases)(Table V).This is similar to the observations of Charanjeet et al (2001)[86] with 90.4% and Vijaya VA et al loc.cit (1999)[80] with 91.4%.The positivity of the H. pylori with the activity status was statistically significant.

We had 22 cases with intestinal metaplasia in our biopsy series.The positivity status for H.pylori in this group was 72.7% (16 cases)(Table V).The positivity status is similar to the observations made by Vijaya VA et al loc.cit (1999)[80] with 78.2% positivity.This goes in contrast to the observations made by Charanjeet et al (2001)[86] in his series on 50 cases, found a low H.pylori positivity of 25 %. We found that intestinal metaplasia was significantly more common in H.pylori positive biopsies. The organisms were not seen in the metaplastic epithelium but were seen in the adjacent gastric mucosa.

We had a significantly large number of cases (33 cases out of 100) presenting with atrophy of the gastric mucosa with with widely spaces pits with villiform mucosa and glands lined by low cuboidal cells with depletion of mucin(Table V).Vatsala et al (2001)[83] had 31 cases reported in her series. Vijaya VA et al loc cit (1999)[80] had a very low number of cases with atrophy in her study population. Analysis of H pylori status in cases of atrophic gastritis, we had a positivity in 23 of our cases (69.6%) .

Many authors have observed that development and extension of atrophic gastritis was more common in H pylori positivity than in negative cases .kohli Y et al (1993)[87],Sakaki et al (2002)[88]and Gilvarry JM et al (1994)[89]. The determinants of atrophy represent a process leading to the loss of glands in the gastric mucosa.This results from impaired epithelial regeneration.The bacteria through its inflammatory mediators induces degeneration of the glandular epithelial cells and also interferes with the regenerating capacity of the glands.

A detailed analysis of the histomorpholgical changes noted in the antral biopsy specimens in cases of H pylori positivity is given in the table VI.

Mucosal Hyperemia and Edema :

Mucosal hyperemia and edema are often endoscopic or pathologic indicators of chemical injury. However, congestion and edema may also be prominent features in H. pylori associated gastritis due to the underlying inflammatory processes .In our study we had observed mucosal edema and congestion in most of our cases

Surface Epithelial Degeneration :

Surface epithelium degeneration is considered a nonspecific response to injury seen, to a variable degree, in all forms of gastritis. However, it is most conspicuous in two particular disorders: chemical

gastritis (resulting from bile reflux, ethanol, or NSAID use) and H. Pylori gastritis.

We studied this particular change in all the biopsies which was stained positive for H pylori .The most common observation we found was the transformation of normal columnar epithelial cell into cuboidal-shaped epithelium along with mucin depletion.The depletion of mucin was obvious in the lining foveolar cells.These changes are found in most of the cases of H. pylori gastritis, even in areas where the organism found to be scanty.

Focal epithelial Regeneration :

We also observed focal epithelial regeneration of the cells with piling up of epithelial cells at the surface of the mucosa in few of our cases, This matches with the observations made by the authors who termed it as epithelial regeneration ,which is made up of multiple cells at the surface of the mucosa ,a well known feature of H. pylori gastritis.

Surface erosion :

We observed superfical epithelial erosions in most of our cases.The erosions were associated with neutrophilic infiltration with areas of edema. H.pylori associated surface erosion endoscopically appear as elevated lesion and microscopically they present with central fibrinod necrosis along with neutrophils and cellular debris and their margin shows both hyperplastic and regenerative changes.

Foveolar Hyperplasia :

In our study we found antral mucosa lined by low cuboidal cells with increase nuclear cytoplasmic ratio with hyperchromatic nuclei and mitotic activity admixed with depletion of mucin .This accounts for the terminology of pit hyperplasia.

Lymphoid Aggregates and Follicles :

In normal person usually gastric mucosa will show small aggregates of lymphoid cell near muscularis mucosae or at the base of lamina propria but in case of H.pylori associated gastritis lymphoid follicles will be seen with germinal centers.

In our series we have observed presence of lymphoid aggregates in most of the cases with H. pylori positivity 56 cases (87.5%)and in many cases we observed formation of prominent lymphoid follicles with prominent germinal centre 50 cases (78.1%). Other studies were concordance with our study they found that lymphoid follicles or aggregates have been detected in virtually all subjects with H. pylori gastritis.So it is well known factor from our study and other study presence of lymphoid follicle is strongly suggest the presence of H.pylori infection.

Intestinal Metaplasia :

We had 22 cases presenting with intestinal metaplasia of which 16 were positive for H.pylori. The bacteria was identified at the adjacent

gastric mucosa and it is negative in the metaplastic epithelium. Several studies have shown that intestinal metaplasia occurs more frequently in patients with H. pylori gastritis.

Atrophy :

In our study we had 33 cases presenting with features of gastric atrophy of which 23 (69.6%) were positive of H.pylori.

Eosinophil Infiltration :

In normal patient few eosinophils usually present in the lamina propria.But in H.pylori associated gastritis there will be mild eosinophil infiltration will be seen along with other inflammatory cells . In our study We had significant infiltration of eosinophils in very few of our cases (12 cases).

Histological Identification of Helicobacter pylori – Comparison of staining Methods :

For the past decades many authors were undergone various investigatory methods for detecting the spiral shape organism in the gastric mucosa and its association with gastrointestinal diseases . Atlast they succeeded with their effort and found that H.pylori organism plays an important role in the etiology of many gastrointestinal diseases. H.pylori has been established as a major cause for chronic gastritis . So its identification and eradictation is significantly important to save the life of the patient form H.pylori asscoicated diseases .so it is in the hands of

pathologist to give accurate diagnosis to help the clinicians and thereby saves the patient . Various techniques were adopted both invasive and noninvasive to detect the organism used including serology,culture, rapid urease test, C13 urea breath test and histology. Among this histological detection of H.pylori is gold standard test compare to the other test . Many investigators followed different staining method to detect the organism such as modified Giemsa, Warthin –Starry, Gimenez, Genta and latest immunohistochemical H pylori antibody stains.In the present study we used two stains for identifying the bacteria in the endoscopic biopsies.We used the conventional Warthin Starry silver stain and Light Green carbol Fuchsin stain for the detection of the bacteria.

Warthin –Starry as able to pick up the bacteria in 57 cases and Light green Carbol Fuchsin stain stained positive for the bacteria in 61cases.Both the stains were positive in 56 cases and the staining pattern was observed by two individual observers without prior information(Table VII&VIII).

We recommend the use of Light Green Carbol Fuchsin stain as an alternate for the conventional Warthin Starry silver stain or it can be used as an additional staining method for the detection , as it is cheaper, less time consuming and detection of the bacteria is easier (pink spiral forms against green background)(Table IX).

Analysis of the Esophageal Lesions :

As a part of our study,the endoscopic appearance of the esophagus was also studied in the process of evaluating the cause for the acid peptic disease.Biopsies were taken from the lesions as and when necessary.

We had esophageal biopsies taken from 76 cases out of the total 100 cases. The various histomorphological changes are summarized in the table X.

In 6 of the cases the esophagus did not show any remarkable pathological changes.

The most common esophageal lesion seen in our series of patients was that of Chronic Reflux Esophagitis (28 cases) (36.8%), followed by chronic non specific esophagitis and others.The case of chronic reflux esophagitis was associated with expansion of the basal zone with elongation of the vascular papillae.This correlates well with the observation by Collins BJ et al (1996)[90] who was of the opinion that this is a very significant histological change in patients with chronic reflux esophagitis and can be taken as a marker for the reflux disease.Balloon degeneration of the epithelial cells is found to be yet another indicator of epithelial cell injury and we had 10 cases withthis feature.Jessurun JY et al (1988)[91] proposed that the presence of these balloon cells indicates epithelial injury.Presence of intraepithelial eosinophils is an additional

indicatorfor reflux disease .Tummala V et al (1987).[92]However only 6 of our cases shows presence of intraepithelial eosinophils.

The next significant esophageal pathology noted was that of Barrett's esophagus in 14 of our cases (18.42%).The most common type of metaplastic epithelium was that of columnar cells resembling gastric mucosal cells. Inflammation was not very significant in the cases of Barrett's esophagus, we had noticed non specific inflammation in 4 cases and ulceration in 2 cases.This is in coherence with the observation made by Petras RE et al (1991)[93]who observed that ulceration and inflammation are non specific changes associated with Barrett's esophagus.The other histological changes in the esophagus included that of Chronic non specific esophagitis and vascular ectasia of esophagus.

SUMMARY AND CONCLUSION

Infection with Helicobacter pylori has been established as the major cause of chronic gastritis and is important in the pathogenesis of other gastroduodenal diseases such as peptic ulcerations, gastric adenocarcinoma and gastric lymphoma.

In view of this significance,an accurate diagnosis of the infection becomes essential to institute eradication treatment in appropriate cases.Endoscopic examination of the gastric and esophageal mucosa becomes mandatory in patients with symptoms of acid peptic disease.Histological diagnosis of gastric lesions along with detection of H pylori becomes a part of the protocol of work up in patients with symptoms of acid peptic disease.

We undertook this study on 145 patients presenting with symptoms of acid peptic disease and found significant endoscopic lesions in 100 patients. They were recruited for the detailed histomorphological study of the gastric mucosa along with the use of two special stains for the detection of H pylori – Warthin Starry and Light Green Carbol Fuchsin stain.The gastric lesions were classified and grade according to the Modified Sydney system of classification.The presence of H pylori was evaluated with two stains by two independent observers.

We had a 64% positivity for H pylori which reveals the common prevalence of the organism in the geographic area.Predominant cases (47) presented with moderate inflammation and activity.The significant histomorpholgical changes were surface epithelial degeneration, focal epithelial regeneration, mucosal hyperemia and edema, mucus depletion,lymphoid aggregates and lymphoid follicles.We had 33 cases presenting with features of atrophy and 22 cases with features of intestinal metaplasia.H.pylori staining was positive in cases with intestinal metaplasia and atrophy.Warthin starry stain was able to pick up the bacteria in 57 cases and Light Green stain picked up the organism in 61 cases. We recommend the use of both the stains in combination in the detection of H pylori. The Light green stain was more economical and cheaper than the conventional silver staining techniques.The patient diagnosed to be positive for H pylori have been instituted antimicrobial therapy and are being followed up frequently.

Histomorphological analysis of esophagus was done in 76 cases and we found the most common histological change was that of chronic reflux esophagitis followed by chronic non specific esophagitis, Barrett’s esophagus and Vascular ectasia.In few of the cases the esophagus was normal.The cases of Barrett’s esophagus are kept under close follow up.

Thus we conclude a thorough clinical examination and history, followed by endoscopic evaluation and methods to detect presence of H pylori organism becomes mandatory in the evaluation of case with symptoms of acid peptic disease.Use of combination of special stains increases the sensitivity of detection of the organism.

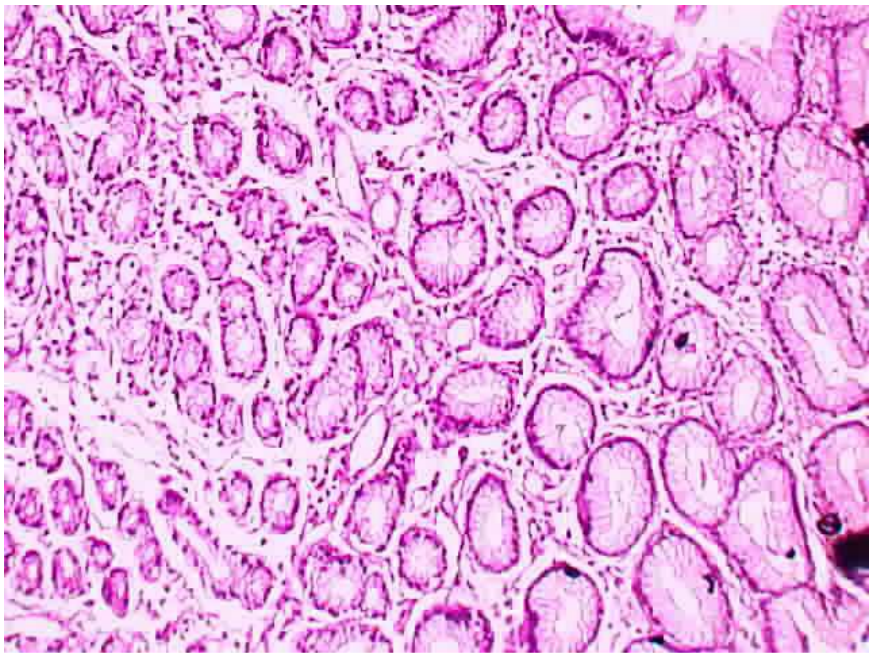


FIG:1 Photomicroscopic picture of gastric antral mucosa shows normal glands(H&E,X100)

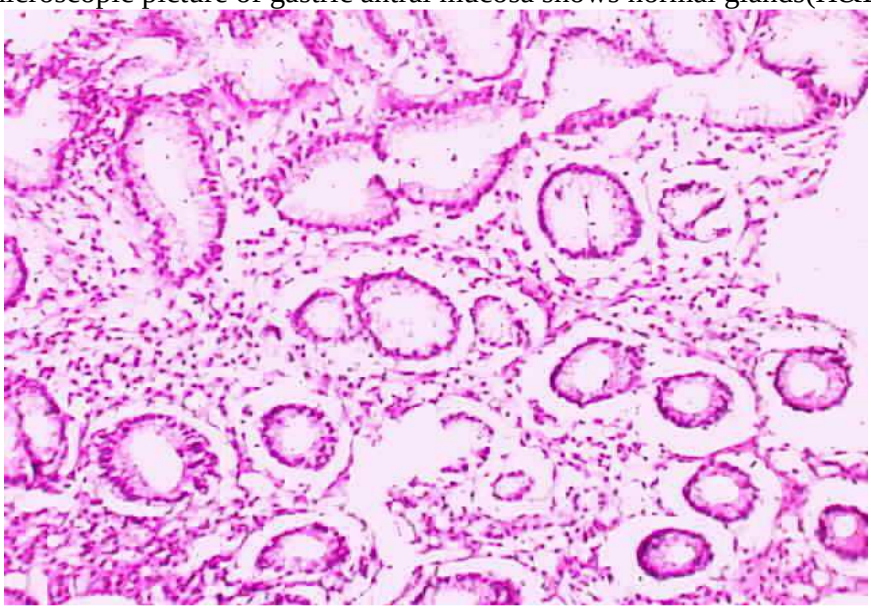


FIG:2 Photomicroscopic picture of gastric antral mucosa shows mild inflammation with mild activity and eosinophils(H&E,X100)

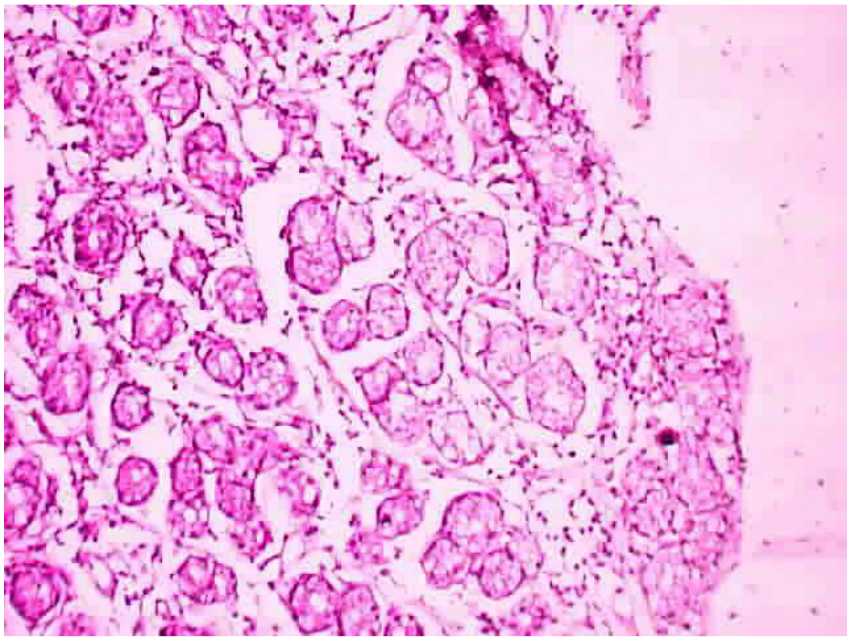


FIG:3 Photomicroscopic picture of gastric antral mucosa shows mild inflammation(H&E,X100)

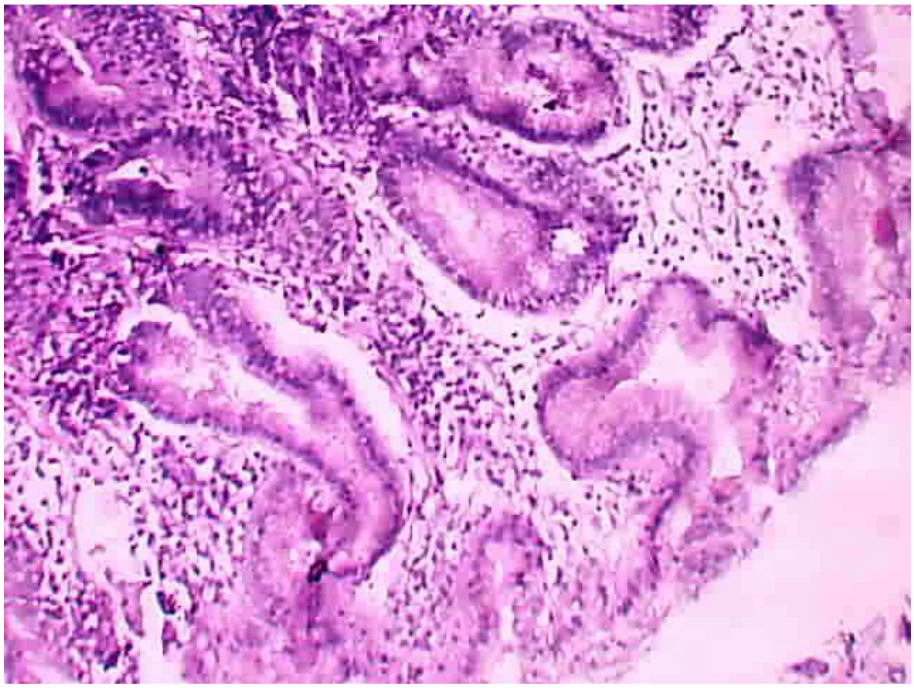


FIG:4 Photomicroscopic picture of gastric antral mucosa shows foveolar pitting with moderate inflammation(H&E,X100)

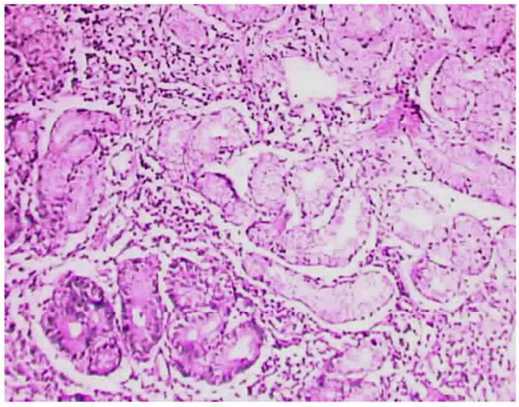


FIG:5 Photomicroscopic picture of gastric antral mucosa shows severe inflammation with moderate activity (H&E,100X)

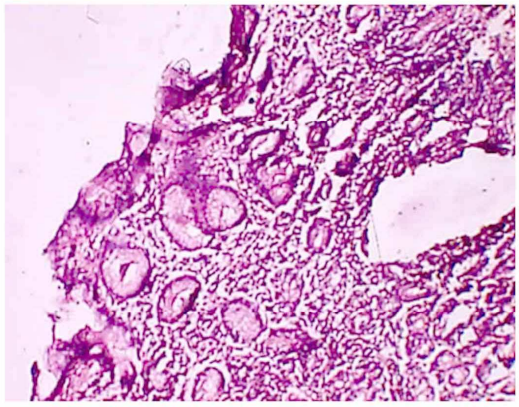


FIG:6 Photomicroscopic picture of gastric antral mucosa shows severe inflammation with lymphoid follicle (H&E,100X)

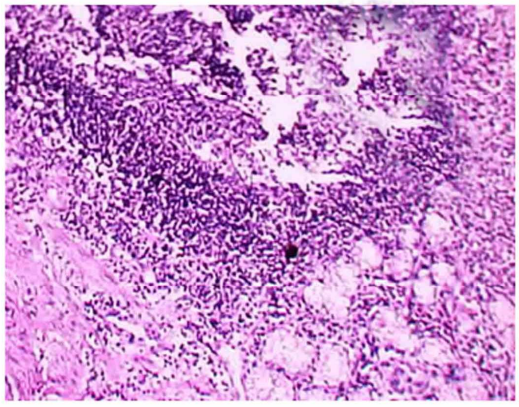


FIG:7 Photomicroscopic picture of gastric antral mucosa shows lymphoid follicle with severe activity. (H&E,100X)

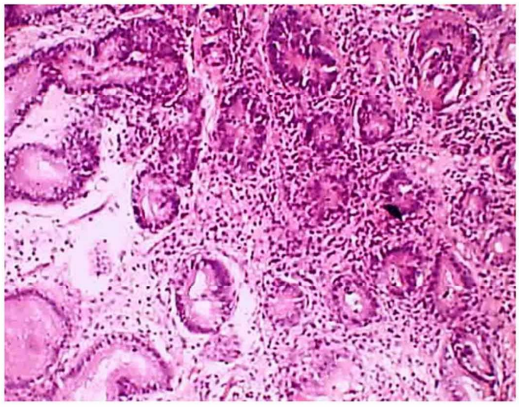


FIG:8 Photomicroscopic picture of gastric antral mucosa shows mild atrophy with severe inflammation (H&E,100X)

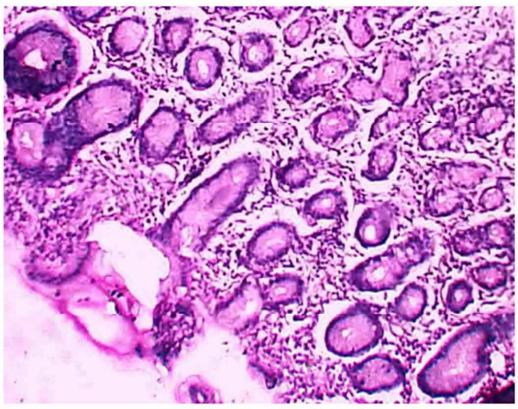


Fig :9 Photomicroscopic picture of gastric antral mucosa shows epithelial erosion with atrophic glands and mild inflammation(H&E,100X)

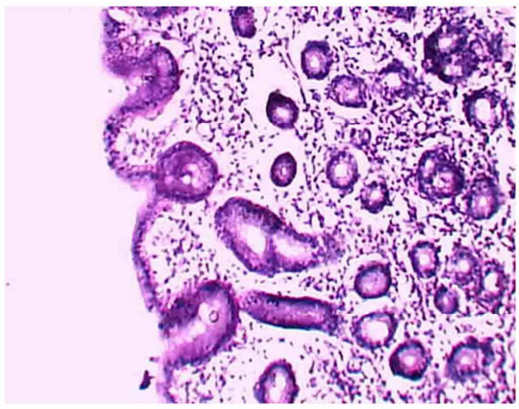


Fig :10 Photomicroscopic picture of gastric antral mucosa shows mucus depletion with moderate atrophic glands and moderate inflammation(H&E,100X).

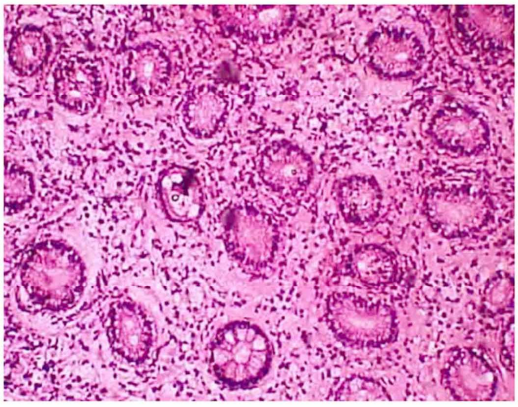


Fig :11 Photomicroscopic picture of gastric antral mucosa shows metaplastic atrophic glands(H&E,100X)

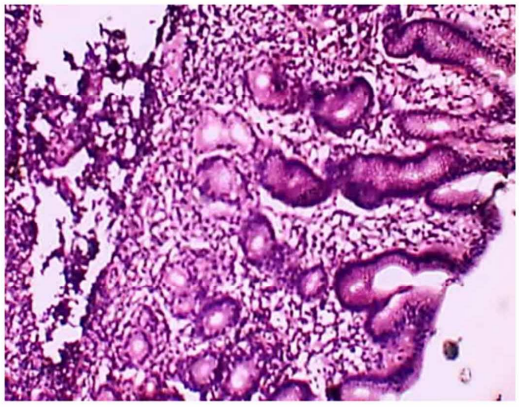


Fig 12: Photomicroscopic picture of gastric antral mucosa shows atrophic glands with lymphoid follicle(H&E,100X)

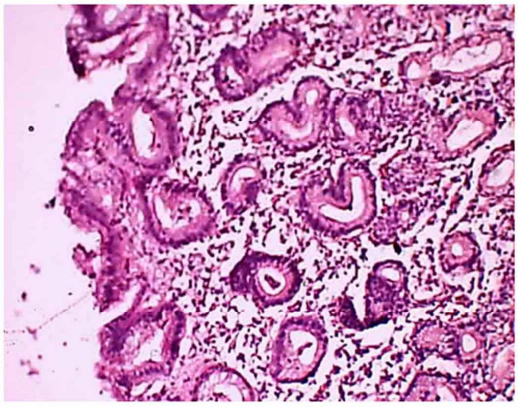


Fig :13 Photomicroscopic picture of gastric antral mucosa shows focal epithelial degeneration With atrophy and mild inflammation(H&E,100X)

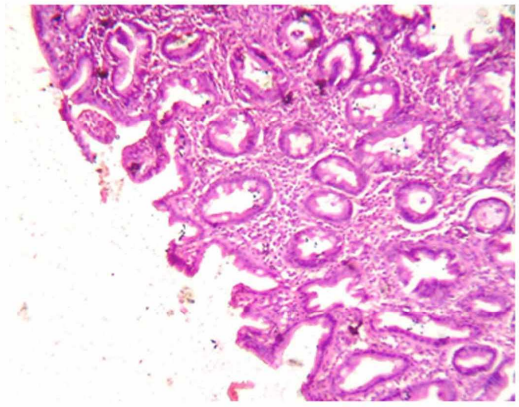


Fig :14 Photomicroscopic picture of gastric antral mucosa shows mild intestinal metaplasia(H&E,100X)

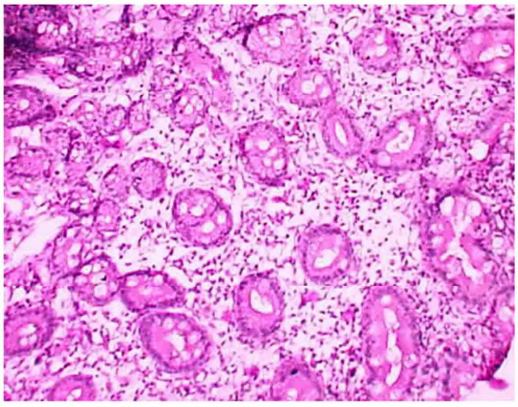


Fig :15 Photomicroscopic picture of gastric antral mucosa shows moderate intestinal metaplasia(H&E,100X)

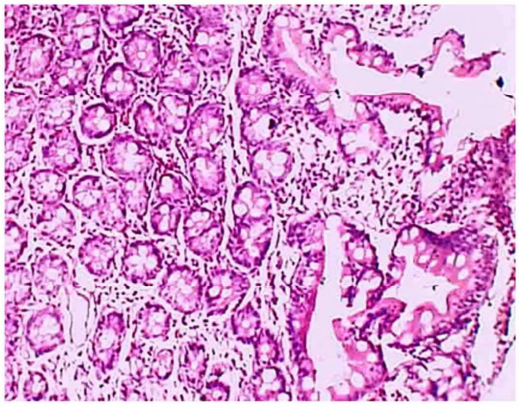


Fig:16 Photomicroscopic picture of gastric antral mucosa shows florid intestinal metaplasia (H&E,100X)

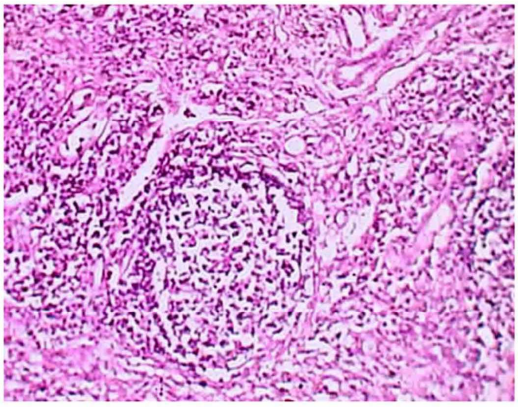


Fig :17 Photomicroscopic picture of gastric antral mucosa shows prominent lymphoid follicle(H&E,100 X)

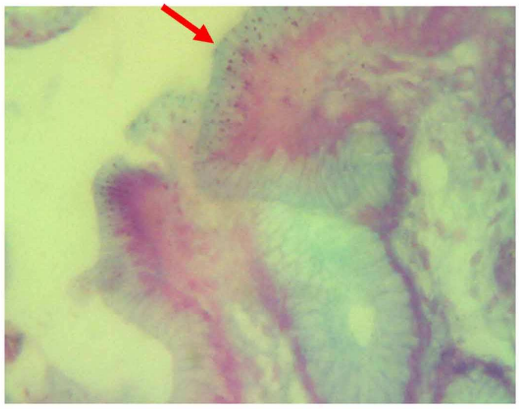


Fig :18 Carbol fuchsin and light green (400X) – H.pylori infection present (Arrow) in the epithelial surface

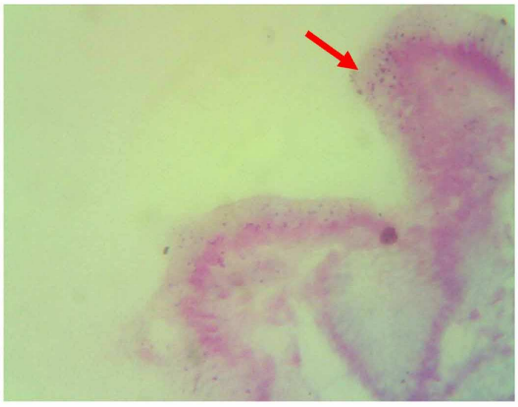


Fig :19 Carbol fuchsin and light green (400X) – H.pylori infection present (Arrow) in the epithelial surface

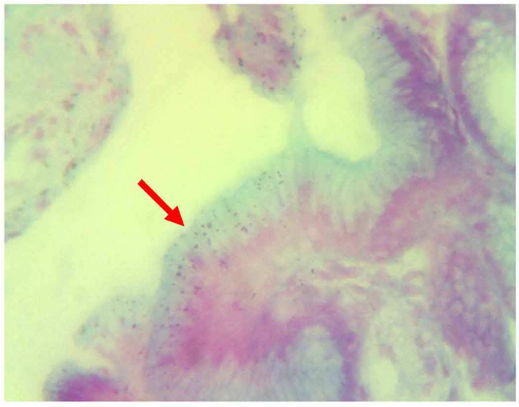


Fig :20 Carbol fuchsin and light green (400X) – H.pylori infection present (Arrow) in the epithelial surface

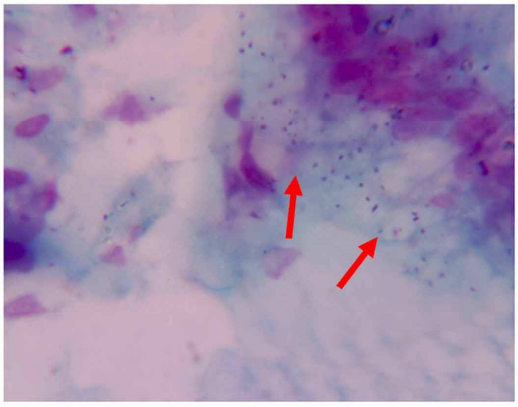


Fig :21 Warthin starry(1000X) – H.pylori infection present (Arrow) in the epithelial surface of the glandular lumen.

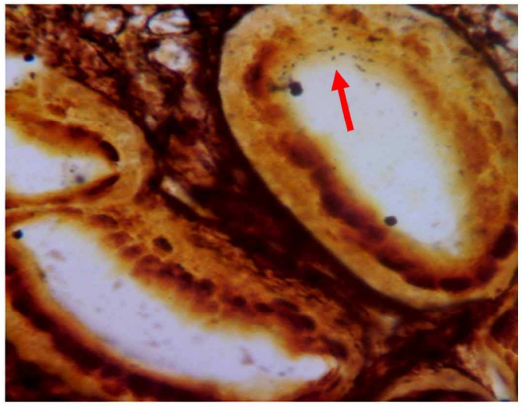


Fig :22 Warthin starry(400 X) – H.pylori infection present (Arrow) in the epithelial surface of the glandular lumen.

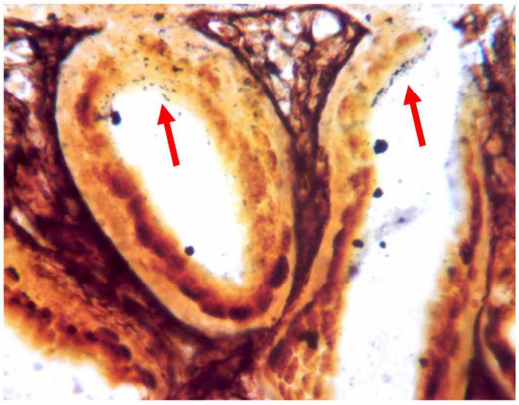


Fig :23 Warthin starry(400X) – H.pylori infection present (Arrow) in the epithelial surface of the glandular lumen.

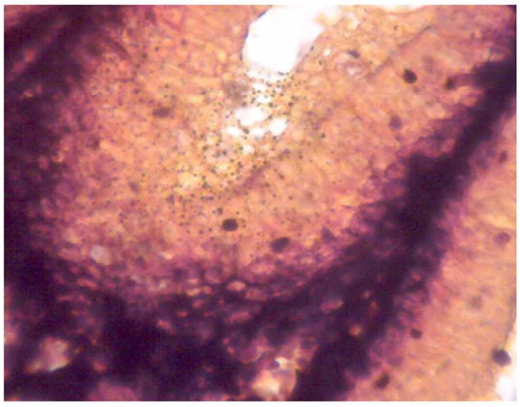


Fig :24 Warthin starry(1000X) – H.pylori infection present in the epithelial surface of the glandular lumen.

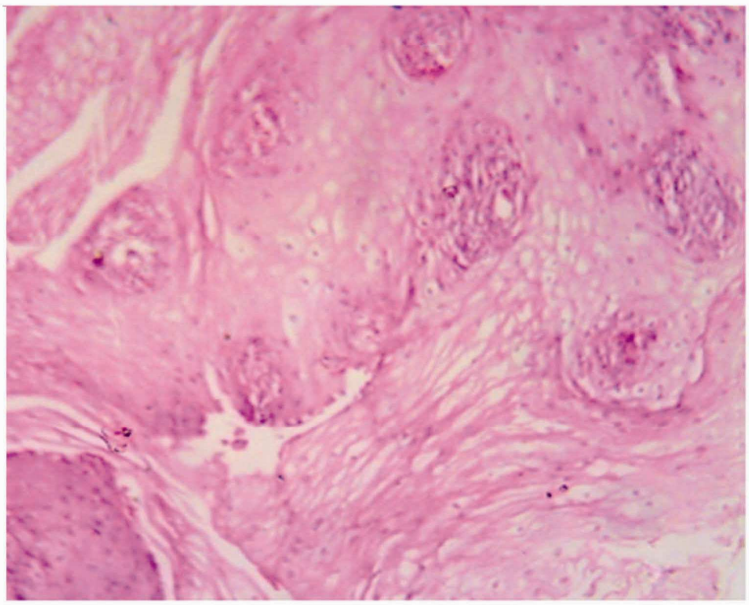


Fig:25 Photomicroscopic picture of esophagus shows mild intraepithelial inflammation (H&E,100X)

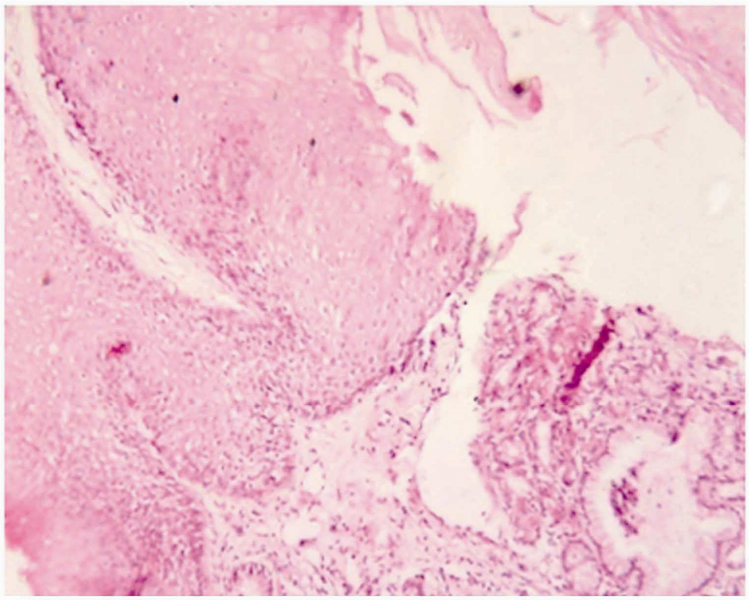


Fig :26 Photomicroscopic picture shows Barrets esophagus (H&E,100X)

S.no	Ref.no	AGE	SEX	SYDNEY SCORING FOR GASTRITIS					H.pylori-WS	H.pylori-CF &LG
				CI	ACTIVITY	IM	ATROPHY	H.pylori		
1	1474/11	41	F	3	2	1	0	POS	POS	POS
2	1475/11	54	F	0	0	1	1	NEG	NEG	NEG
3	1476/11	40	M	3	1	0	1	POS	POS	POS
4	1477/11	28	M	2	0	2	0	POS	POS	POS
5	1478/11	34	M	3	2	0	1	POS	POS	POS
6	1479/11	26	M	3	0	0	0	POS	POS	POS
7	1492/11	36	F	1	0	0	0	NEG	NEG	NEG
8	1493/11	61	F	2	2	0	1	POS	POS	POS
9	1494/11	68	M	1	0	0	0	NEG	NEG	NEG
10	1495/11	72	M	0	0	0	1	NEG	NEG	NEG
11	1496/11	34	M	3	2	0	0	POS	POS	POS
12	1550/11	44	M	2	2	0	0	POS	POS	POS
13	1551/11	69	F	2	1	0	0	POS	NEG	POS
14	1553/11	40	M	2	0	1	2	NEG	NEG	NEG
15	1554/11	34	M	3	2	1	1	POS	POS	POS
16	1555/11	39	M	2	2	0	0	POS	POS	POS
17	1556/11	63	M	2	2	1	2	POS	POS	POS
18	1557/11	53	M	2	1	0	3	POS	POS	POS
19	1558/11	39	F	1	0	0	0	NEG	NEG	NEG
20	1579/11	76	M	1	0	0	1	NEG	NEG	NEG
21	1580/11	36	F	2	0	0	0	NEG	NEG	NEG
22	1581/11	43	M	3	2	0	0	POS	POS	POS
23	1611/11	11	M	0	1	0	0	NEG	NEG	NEG
24	1612/11	44	M	2	0	0	1	POS	POS	POS
25	1613/11	38	M	2	1	1	2	POS	POS	POS
26	1614/11	45	F	1	0	0	0	NEG	NEG	NEG
27	1615/11	32	F	2	1	0	0	POS	POS	POS
28	1616/11	84	M	1	0	0	0	NEG	NEG	NEG
29	1660/11	24	M	1	0	0	0	NEG	NEG	NEG
30	1662/11	72	M	1	0	0	1	NEG	NEG	NEG
31	1666/11	54	F	2	1	0	1	POS	POS	POS
32	1667/11	62	F	3	2	1	2	POS	POS	POS

S.no	Ref.no	AGE	SEX	SYDNEY SCORING FOR GASTRITIS					H.pylori-WS	H.pylori-CF &LG
				CI	ACTIVITY	IM	ATROPHY	H.pylori		
33	1697/11	28	M	2	2	0	0	POS	POS	POS
34	1699/11	45	M	3	0	0	3	NEG	NEG	NEG
35	1701/11	23	M	0	1	0	0	NEG	NEG	NEG
36	1742/11	52	M	2	1	0	1	POS	POS	POS
37	1745/11	68	M	1	0	1	1	NEG	NEG	NEG
38	1746/11	39	F	3	2	0	0	POS	POS	POS
39	1747/11	46	F	1	0	0	1	POS	NEG	POS
40	1748/11	44	M	2	2	0	0	POS	POS	POS
41	1749/11	62	M	3	1	1	1	POS	POS	POS
42	1750/11	69	F	1	0	0	1	NEG	NEG	NEG
43	1764/11	74	M	2	1	1	1	POS	POS	POS
44	1802/11	40	M	3	0	0	1	NEG	NEG	NEG
45	1805/11	33	M	2	0	1	1	POS	POS	POS
46	1807/11	44	F	1	0	0	0	NEG	NEG	NEG
47	1808/11	62	M	2	0	0	1	NEG	NEG	NEG
48	1838/11	38	M	2	1	0	0	POS	POS	POS
49	1839/11	24	M	2	1	0	0	POS	NEG	POS
50	1840/11	24	M	1	0	0	0	NEG	NEG	NEG
51	1841/11	36	M	2	0	1	1	POS	POS	POS
52	3764/12	23	F	2	1	1	0	POS	POS	NEG
53	3765/12	48	F	2	3	0	0	POS	POS	POS
54	3766/12	25	M	0	1	0	0	NEG	NEG	NEG
55	3767/12	25	M	3	1	1	0	POS	POS	POS
56	3768/12	22	M	2	2	0	0	POS	NEG	POS
57	3769/12	24	M	1	0	0	0	NEG	NEG	NEG
58	3770/12	30	F	3	1	0	0	POS	POS	POS
59	3808/12	23	M	0	0	1	0	NEG	NEG	NEG
60	3810/12	27	M	2	1	0	0	POS	POS	POS
61	3812/12	16	F	0	1	0	0	NEG	NEG	NEG
62	3833/12	68	M	1	1	0	2	POS	POS	POS
63	3834/12	33	F	1	0	0	0	NEG	NEG	NEG
64	3835/12	41	F	0	1	0	0	NEG	NEG	NEG

S.no	Ref.no	AGE	SEX	SYDNEY SCORING FOR GASTRITIS					H.pylori-WS	H.pylori-CF &LG
				CI	ACTIVITY	IM	ATROPHY	H.pylori		
65	3836/12	44	F	1	1	0	0	POS	POS	POS
66	3857/12	32	M	2	2	1	0	POS	POS	POS
67	3858/12	29	M	0	0	2	0	NEG	NEG	NEG
68	3860/12	27	M	2	2	1	0	POS	POS	NEG
69	4648/12	42	F	1	2	1	0	POS	POS	POS
70	4649/12	34	M	1	0	1	0	NEG	NEG	NEG
71	4651/12	52	M	1	2	0	2	POS	NEG	POS
72	7117/12	37	F	2	1	0	0	POS	NEG	POS
73	7118/12	27	F	2	1	0	0	POS	POS	POS
74	7120/12	42	M	2	3	0	0	POS	POS	POS
75	7144/12	29	F	2	0	0	0	NEG	NEG	NEG
76	7150/12	18	F	2	1	0	0	POS	NEG	POS
77	7151/12	52	M	2	1	0	0	POS	POS	NEG
78	7152/12	42	M	2	1	0	0	POS	POS	POS
79	7153/12	74	M	2	1	0	2	POS	POS	POS
80	7185/12	24	M	2	0	0	0	NEG	NEG	NEG
81	7186/12	31	F	2	2	0	0	POS	POS	POS
82	7187/12	38	M	2	2	0	0	POS	POS	POS
83	7268/12	63	M	2	2	0	2	POS	POS	POS
84	7271/12	39	F	2	3	0	0	POS	POS	POS
85	7272/12	53	M	2	3	0	0	POS	POS	POS
86	7347/12	32	M	0	1	0	0	NEG	NEG	NEG
87	7348/12	76	M	2	1	0	2	POS	POS	POS
88	7374/12	72	M	2	2	0	1	POS	POS	POS
89	7375/12	42	M	2	2	0	0	POS	POS	POS
90	7376/12	38	M	2	3	0	0	POS	POS	POS
91	7377/12	42	F	3	2	0	0	POS	POS	POS
92	7511/12	17	M	1	0	0	0	NEG	NEG	NEG
93	7512/12	11	M	0	1	0	0	NEG	NEG	NEG
94	7518/12	38	M	1	1	0	0	NEG	NEG	NEG
95	7519/12	45	F	1	3	0	0	POS	POS	POS
96	7520/12	84	M	1	2	0	0	POS	POS	POS

S.no	Ref.no	AGE	SEX	SYDNEY SCORING FOR GASTRITIS					H.pylori-WS	H.pylori-CF &LG
				CI	ACTIVITY	IM	ATROPHY	H.pylori		
97	7521/12	74	M	1	2	0	0	POS	POS	POS
98	7587/12	54	M	1	0	0	0	NEG	NEG	NEG
99	7588/12	54	M	1	1	1	0	POS	POS	POS
100	7589/12	66	M	2	3	0	1	POS	POS	POS

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APPENDIX

DEPARTMENT OF PATHOLOGY, TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

PROFORMA

NAME : BIOPSY No. :

AGE : OP/IP No. :

SEX : DEPARTMENT :

OCCUPATION :

ADDRESS :

1. CLINICAL DETAILS :

a) CHIEF COMPLAINTS :

b) PAST HISTORY :

c) FAMILY HISTORY :

d) PERSONAL HISTORY :

e) GENERAL EXAMINATION :

f) SYSTEMIC EXAMINATION :

CVS :

RS :

CNS :

PA :

2. INVESTIGATIONS :

a) ROUTINE : Haemoglobin, Total Leucocyte count

b) UPPER GASTROINTESTINAL ENDOSCOPY:

3. CLINICAL DIAGNOSIS :

4. HISTOPATHOLOGICAL EXAMINATION :

a) MACROSCOPY:

b) MICROSCOPY:

c) SPECIAL STAINS:

5. HISTOPATHOLOGICAL DIAGNOSIS :

KEY TO MASTER CHART

M	-	Male
F	-	Female
1	-	Mild
2	-	Moderate
3	-	Severe
CF	-	Chronic Inflammation
IM	-	Intestinal metaplasia
H.pylori	-	Helicobacter pylori
POS	-	Positive
NEG	-	Negative
WS	-	Warthin starry
CF & LG	-	Carbol fuchsin and light green